NUCLEIC ACID SEQUENCES AND METHODS OF USE FOR THE PRODUCTION OF PLANTS WITH MODIFIED POLYUNSATURATED FATTY ACIDS

FIELD OF THE INVENTION

The present invention is directed to nucleic acid molecules and nucleic acid constructs, and other agents associated with fatty acid synthesis. Moreover, the present invention is directed to plants incorporating such agents where the plants exhibit altered ratios of saturated and unsaturated fats. In particular, the present invention is directed to plants incorporating such agents where the plants exhibit altered ratios of monounsaturated to polyunsaturated fatty acids.

BACKGROUND

Plant oils are used in a variety of applications. Novel vegetable oil compositions and improved means to obtain oil compositions, from biosynthetic or natural plant sources, are needed. Depending upon the intended oil use, various different fatty acid compositions are desired.

Higher plants appear to synthesize fatty acids via a common metabolic pathway -- the fatty acid synthetase (FAS) pathway. In developing seeds, where fatty acids are attached to glycerol backbones, forming triacylglycerides, for storage as a source of energy for further germination, the FAS pathway is located in the plastids. The first committed step is the formation of acetyl-ACP (acyl carrier protein) from acetyl-CoA and ACP catalyzed by the enzyme, acetyl-CoA:ACP transacylase (ATA). Elongation of acetyl-ACP to 16- and 18-carbon fatty acids involves the cyclical action of the following sequence of reactions: condensation with a two-carbon unit from malonyl-ACP to form a β-ketoacyl-ACP (β-ketoacyl-ACP synthase), reduction of the keto-function to an alcohol (β-ketoacyl-ACP reductase), dehydration to form an enoyl-ACP (β-ketoacyl-ACP dehydratase), and finally reduction of the enoyl-ACP to form the elongated saturated acyl-ACP (enoyl-ACP reductase). β-ketoacyl-ACP synthase I catalyzes elongation from C4:0 up to palmitoyl-ACP (C16:0), whereas β-ketoacyl-ACP synthase II

catalyzes the final elongation to stearoyl-ACP (C18:0). Common plant unsaturated fatty acids, such as oleic, linoleic and linolenic acids found in storage triacylglycerides, originate from the desaturation of stearoyl-ACP to form oleoyl-ACP (C18:1) in a reaction catalyzed by a soluble plastid Δ-9 desaturase (also often referred to as "stearoyl-ACP desaturase"). Molecular oxygen is required for desaturation in which reduced ferredoxin serves as an electron co-donor. Additional desaturation is effected sequentially by the actions of membrane bound Δ-12 desaturase and Δ-15 desaturase. These "desaturases" thus create polyunsaturated fatty acids.

Obtaining nucleic acid sequences capable of producing a phenotypic result in FAS, desaturation and/or incorporation of fatty acids into a glycerol backbone to produce an oil is subject to various obstacles including but not limited to the identification of metabolic factors of interest, choice and characterization of an enzyme source with useful kinetic properties, purification of the protein of interest to a level which will allow for its amino acid sequencing, utilizing amino acid sequence data to obtain a nucleic acid sequence capable to use as a probe to retrieve the desired DNA sequence, and the preparation of constructs, transformation and analysis of the resulting plants.

Thus, additional nucleic acid targets and methods for modifying fatty acid compositions are needed. In particular, constructs and methods to produce a range of different fatty acid compositions are needed.

SUMMARY OF THE INVENTION

The present invention provides a substantially purified nucleic acid molecule comprising a nucleic acid sequence with at least 70% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 4, complements thereof, and fragments of either.

Further provided by the present invention are a nucleic acid molecule comprising at least 15 consecutive nucleotides of a nucleic acid molecule having the sequence of SEQ ID NO:12; a nucleic acid molecule comprising at least 15 consecutive nucleotides of a nucleic acid molecule having the sequence of SEQ ID NO:13; a nucleic acid molecule comprising at least 15 consecutive nucleotides of a nucleic acid molecule having the sequence of SEQ ID NO:14; and a

nucleic acid molecule comprising at least 15 consecutive nucleotides of a nucleic acid molecule having the sequence of SEQ ID NO:4.

Also provided by the present invention is a recombinant nucleic acid molecule comprising as operably linked components: (A) a promoter that functions in a plant cell to cause production of an mRNA molecule; and (B) a nucleic acid sequence that hybridizes under high stringency conditions to a nucleic acid sequence selected from the group consisting of SEQ ID NO:12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 4, complements thereof, and fragments of either.

Further provided by the present invention is a transformed soybean plant having a nucleic acid molecule that comprises (a) a first promoter operably linked to a first nucleic acid molecule having a first nucleic acid sequence that has 85% or greater identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:2, complements thereof, and fragments of either, and (b) a second nucleic acid molecule having a second nucleic acid sequence that has 85% or greater identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOs:4 through SEQ ID NO:14, complements thereof, and fragments of either, wherein the second nucleic acid molecule is operably linked to the first promoter in a polycistronic configuration or to a second promoter.

Further provided by the present invention is a transformed soybean plant comprising a double- strand RNAi construct where a first promoter is operably linked to a first nucleic acid molecule, having a first nucleic acid sequence that has 85% or greater identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:2, complements thereof, and fragments of either, wherein a second nucleic acid molecule having a second nucleic acid sequence that has 85% or greater identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4 through SEQ ID NO:14, complements thereof, and fragments of either, is operably linked to the first nucleic acid molecule.

Also provided by the present invention is a transformed soybean plant comprising a double- strand RNAi construct where a first promoter is operably linked to a first nucleic acid molecule, having a first nucleic acid sequence that has 85% or greater identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:2,

complements thereof, and fragments of either, wherein a second nucleic acid molecule having a second nucleic acid sequence that has 85% or greater identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4 through SEQ ID NO:14, complements thereof, and fragments of either is operably linked to a second promoter in a dsRNAi configuration.

Also provided by the present invention is a transformed soybean plant having two or more nucleic acid molecules wherein each nucleic acid molecule is operably linked to a promoter and wherein each nucleic acid molecule has a nucleic acid sequence that has 85% or greater identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 2, 4-14, complements thereof, and fragments of either.

The present invention provides a transformed soybean plant, wherein the level of a transcript encoded by a gene selected from the group consisting of *FAD2-1A*, *FAD2-1B*, *FAD2-2B*, *FAD3-1A*, *FAD3-1B*, *FAD3-1C* is selectively reduced while leaving the level of a transcript encoded by a different gene selected from the group consisting of *FAD2-1A*, *FAD2-1B*, *FAD2-2B*, *FAD3-1A*, *FAD3-1B*, *FAD3-1C* at least partially unaffected.

The present invention also provides a method of producing a soybean plant having a seed with reduced linolenic acid content comprising: transforming a soybean plant with a nucleic acid molecule that comprises (a) a first promoter operably linked to a first nucleic acid molecule having a first nucleic acid sequence that has 85% or greater identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:2, complements thereof, and fragments of either, and (b) a second nucleic acid molecule having a second nucleic acid sequence that has 85% or greater identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4 through SEQ ID NO:14, complements thereof, and fragments of either, wherein the second nucleic acid molecule is operably linked to the first promoter or a second promoter; and growing said plant, wherein said plant produces seed with less linolenic acid than a plant having a similar genetic background but lacking said nucleic acid molecule.

The present invention also provides a method of producing a soybean plant having a seed with increased oleic acid content comprising: transforming a soybean plant with a nucleic acid molecule that comprises (a) a promoter operably linked to a first nucleic acid molecule having a first nucleic acid sequence that has 85% or greater identity to a nucleic acid sequence selected

from the group consisting of SEQ ID NO:1 through SEQ ID NO:2, complements thereof, and fragments of either, and (b) a second nucleic acid molecule having a second nucleic acid sequence that has 85% or greater identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4 through SEQ ID NO:14, complements thereof, and fragments of either, wherein the second nucleic acid molecule is operably linked to the first promoter or a second promoter; and growing said plant, wherein said plant produces seed with more oleic acid than a plant having a similar genetic background but lacking said nucleic acid molecule.

The present invention also provides a method of producing a plant having a seed with a modified oil composition comprising: transforming a plant with a nucleic acid molecule that comprises, as operably linked components, a first promoter and a first nucleic acid molecule having a first nucleic acid sequence that has 85% or greater identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 4 through 14, complements thereof, and fragments of either; and, growing said plant, wherein said plant produces seed with a modified oil composition compared to a plant having a similar genetic background but lacking said nucleic acid molecule.

The present invention further provides a method of producing a plant having a seed with an altered ratio of monounsaturated to polyunsaturated fatty acids comprising: transforming a plant with a construct that comprises, as operably linked components, two or more nucleic acid molecules, each having a nucleic acid sequence that has 85% or greater identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 4 through 14, complements thereof, and fragments of either, wherein each nucleic acid molecule is operably linked to a promoter; and, growing said plant, wherein said plant produces seed with an altered ratio of monounsaturated to polyunsaturated fatty acids compared to a plant having a similar genetic background but lacking said two or more nucleic acid molecules.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic of construct pCGN5468.

Figure 2 is a schematic of construct pCGN5469.

Figure 3 is a schematic of construct pCGN5471.

Figure 4 is a schematic of construct pCGN5485.

Figure 5 is a schematic of construct pCGN5486.

Figure 6 is a schematic of construct pCGN5462.

Figure 7 is a schematic of construct pCGN5466.

Figure 8 is a schematic of construct pCGN5464.

Figure 9 is a schematic of construct pCGN5473.

Figure 10 is a schematic of construct pMON68521.

Figure 11 is a schematic of construct pMON68519.

Figure 12 is a schematic of construct pCGN5455.

Figure 13 is a schematic of construct pCGN5459.

DETAILED DESCRIPTION OF THE INVENTION

Description of the Nucleic Acid Sequences

SEQ ID NO:1 sets forth a nucleic acid sequence of a *FAD2-1A* intron 1.

SEQ ID NO:2 sets forth a nucleic acid sequence of a *FAD2-1B* intron 1.

SEQ ID NO:3 sets forth a nucleic acid sequence of a partial *FAD2-2* genomic clone.

SEQ ID NO:4 sets forth a nucleic acid sequence of a *FAD2-2*B intron 1.

SEQ ID NO:5 sets forth a nucleic acid sequence of a *FAD3-1A* intron 1.

SEQ ID NO:6 sets forth a nucleic acid sequence of a *FAD3-1A* intron 2.

SEQ ID NO:7 sets forth a nucleic acid sequence of a *FAD3-1A* intron 3A.

SEQ ID NO:8 sets forth a nucleic acid sequence of a *FAD3-1A* intron 4.

SEQ ID NO:9 sets forth a nucleic acid sequence of a *FAD3-1A* intron 5.

SEQ ID NO:10 sets forth a nucleic acid sequence of a *FAD3-1A* intron 3B.

SEQ ID NO:11 sets forth a nucleic acid sequence of a *FAD3-1A* intron 3C.

SEQ ID NO:12 sets forth a nucleic acid sequence of a *FAD3-1B* intron 3C.

SEQ ID NO:13 sets forth a nucleic acid sequence of a *FAD3-1B* intron 4.

SEQ ID NO:14 sets forth a nucleic acid sequence of a *FAD3-1C* intron 4.

SEQ ID NO:15 sets forth a cDNA sequence of a *FAD2-1A* gene sequence.

SEQ ID NOs:16 and 17 set forth nucleic acid sequences of *FAD2-1A* PCR primers.

SEQ ID NO:18 sets forth a nucleic acid sequence of a partial *FAD2-1A* genomic clone.

SEQ ID NO:19 sets forth a nucleic acid sequence of a partial *FAD2-1B* genomic clone.

SEQ ID NOs:20 and 21 set forth nucleic acid sequences of *FAD3-1A* PCR primers.

SEQ ID NO:22 sets forth a nucleic acid sequence of a *FAD2-1B* promoter.

SEQ ID NO:23 sets forth a nucleic acid sequence of a partial *FAD3-1A* genomic clone.

SEQ ID NOs:24 through 39 set forth nucleic acid sequences of PCR primers.

Definitions

As used herein, the term "gene" is used to refer to the nucleic acid sequence that encompasses the 5' untranslated region, including promoter region, associated with the expression of the gene product, any intron and exon regions and 5' or 3' untranslated regions associated with the expression of the gene product.

As used herein, a "*FAD2*", "Δ12 desaturase" or "omega-6 desaturase" gene is a gene that encodes an enzyme capable of catalyzing the insertion of a double bond into a fatty acyl moiety at the twelfth position counted from the carboxyl terminus.

When referring to proteins and nucleic acids herein, the use of plain capitals, *e.g.*, "FAD2", indicates a reference to an enzyme, protein, polypeptide, or peptide, and the use of italicized capitals, *e.g.*, "*FAD2*", indicates a reference to nucleic acids, including without limitation genes, cDNAs, and mRNAs.

As used herein the terminology "*FAD2-1*" is used to refer to a *FAD2* gene that is naturally expressed in a specific manner in seed tissue.

As used herein the terminology "*FAD2-2*" is used to refer a *FAD2* gene that is (a) a different gene from a *FAD2-1* gene and (b) is naturally expressed in multiple tissues, including the seed.

As used herein, a "*FAD3*", "Δ15 desaturase" or "omega-3 desaturase" gene is a gene that encodes an enzyme capable of catalyzing the insertion of a double bond into a fatty acyl moiety at the fifteenth position counted from the carboxyl terminus.

As used herein the terminology "*FAD3-1*" is used to refer a *FAD3* gene that is naturally expressed in multiple tissues, including the seed.

As used herein the capital letter that follows the gene terminology (A, B, C) is used to designate the family member, *i.e. FAD2-1A* is a different gene family member from *FAD2-1B*.

As used herein, a "mid-oleic soybean seed" is a seed having between 50% and 75% oleic acid present in the oil composition of the seed.

As used herein, a "high oleic soybean seed" is a seed with oil having greater than 75% oleic acid present in the oil composition of the seed.

The term "non-coding" refers to sequences of nucleic acid molecules that do not encode part or all of an expressed protein. Non-coding sequences include but are not limited to introns, promoter regions, 3' untranslated regions, and 5' untranslated regions.

The term "intron" as used herein refers to the normal sense of the term as meaning a segment of nucleic acid molecules, usually DNA, that does not encode part of or all of an expressed protein, and which, in endogenous conditions, is transcribed into RNA molecules, but which is spliced out of the endogenous RNA before the RNA is translated into a protein.

The term "exon" as used herein refers to the normal sense of the term as meaning a segment of nucleic acid molecules, usually DNA, that encodes part of or all of an expressed protein.

As used herein, a promoter that is "operably linked" to one or more nucleic acid sequences is capable of driving expression of one or more nucleic acid sequences, including multiple coding or non-coding nucleic acid sequences arranged in a polycistronic configuration.

A "polycistronic gene" or "polycistronic mRNA" is any gene or mRNA that contains transcribed nucleic acid sequences which correspond to nucleic acid sequences of more than one gene targeted for expression. It is understood that such polycistronic genes or mRNAs may contain sequences that correspond to introns, 5'UTRs, 3'UTRs, or combinations thereof, and that a recombinant polycistronic gene or mRNA might, for example without limitation, contain sequences that correspond to one or more UTRs from one gene and one or more introns from a second gene.

As used herein, the term complement of a nucleic acid sequence refers to the complement of the sequence along its complete length.

As used herein, any range set forth is inclusive of the end points of the range unless otherwise stated.

Agents

The agents of the invention will preferably be "biologically active" with respect to either a structural attribute, such as the capacity of a nucleic acid molecule to hybridize to another nucleic acid molecule, or the ability of a protein to be bound by an antibody (or to compete with another molecule for such binding). Alternatively, such an attribute may be catalytic and thus involve the capacity of the agent to mediate a chemical reaction or response. The agents will preferably be "substantially purified." The term "substantially purified," as used herein, refers to a molecule separated from substantially all other molecules normally associated with it in its native environmental conditions. More preferably a substantially purified molecule is the predominant species present in a preparation. A substantially purified molecule may be greater than 60% free, greater than 75% free, preferably greater than 90% free, and most preferably greater than 95% free from the other molecules (exclusive of solvent) present in the natural mixture. The term "substantially purified" is not intended to encompass molecules present in their native environmental conditions.

The agents of the invention may also be recombinant. As used herein, the term "recombinant" means any agent (*e.g.*, including but limited to DNA, peptide), that is, or results, however indirectly, from human manipulation of a nucleic acid molecule.

It is understood that the agents of the invention may be labeled with reagents that facilitate detection of the agent (*e.g.*, fluorescent labels, Prober *et al.*, *Science 238*:336-340 (1987); Albarella *et al.*, EP 144914; chemical labels, Sheldon *et al.*, U.S. Patent 4,582,789; Albarella *et al.*, U.S. Patent 4,563,417; modified bases, Miyoshi *et al.*, EP 119448).

Nucleic Acid Molecules

Agents of the invention include nucleic acid molecules. In an aspect of the present invention, the nucleic acid molecule comprises a nucleic acid sequence, which when introduced into a cell or organism, is capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD2* or *FAD3* gene while leaving the level of a protein and/or transcript encoded by a second *FAD2* or *FAD3* gene partially unaffected. In a preferred aspect of the present

invention, the nucleic acid molecule comprises a nucleic acid sequence, which when introduced into a cell or organism, is capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD2* or *FAD3* gene while leaving the level of a protein and/or transcript encoded by a second *FAD2* or *FAD3* gene substantially unaffected. In a highly preferred aspect of the present invention, the nucleic acid molecule comprises a nucleic acid sequence, which when introduced into a cell or organism, is capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD2* or *FAD3* gene while leaving the level of a protein and/or transcript encoded by a second *FAD2* or *FAD3* gene essentially unaffected.

In a preferred aspect, the capability of a nucleic acid molecule to selectively reduce the level of a gene relative to another gene is carried out by a comparison of levels of mRNA transcripts. In another preferred aspect of the present invention, the nucleic acid molecule of the invention comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1 through 15, 18, 19, 22, 23, complements thereof, and fragments of either. In another preferred aspect of the present invention the nucleic acid molecule of the invention comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:16, 17, 20, 21, 24 through 39, complements thereof, and fragments of either.

In one aspect of the present invention the nucleic acids of the present invention are said to be introduced nucleic acid molecules. A nucleic acid molecule is said to be "introduced" if it is inserted into a cell or organism as a result of human manipulation, no matter how indirect. Examples of introduced nucleic acid molecules include, but are not limited to, nucleic acids that have been introduced into cells via transformation, transfection, injection, and projection, and those that have been introduced into an organism via methods including, but not limited to, conjugation, endocytosis, and phagocytosis. The cell or organism can be, or can be derived from, a plant, plant cell, algae cell, algae, fungal cell, fungus, or bacterial cell.

As used herein, "essentially unaffected" refers to a level of an agent such as a protein or mRNA transcript that is either not altered by a particular event or altered only to an extent that does not affect the physiological function of that agent. In a preferred aspect, the level of the agent that is essentially unaffected is within 20%, more preferably within 10%, and even more

preferably within 5% of the level at which it is found in a cell or organism that lacks a nucleic acid molecule capable of selectively reducing another agent.

As used herein, "substantially unaffected" refers to a level of an agent such as a protein or mRNA transcript in which the level of the agent that is substantially unaffected is within 49%, more preferably within 35%, and even more preferably within 24% of the level at which it is found in a cell or organism that lacks a nucleic acid molecule capable of selectively reducing another agent.

As used herein, "partially unaffected" refers to a level of an agent such as a protein or mRNA transcript in which the level of the agent that is partially unaffected is within 80%, more preferably within 65%, and even more preferably within 50% of the level at which it is found in a cell or organism that lacks a nucleic acid molecule capable of selectively reducing another agent.

As used herein, "a selective reduction" of an agent such as a protein or mRNA is relative to a cell or organism lacking a nucleic acid molecule capable of selectively reducing the agent. In a preferred aspect, the level of the agent is selectively reduced by at least 50%, preferably at least more than 75%, and even more preferably by at least more than 90% or 95%.

When levels of an agent are compared, such a comparison is preferably carried out between organisms with a similar genetic background. In a preferred aspect, a similar genetic background is a background where the organisms being compared share 50% or greater of their nuclear genetic material. In a more preferred aspect a similar genetic background is a background where the organisms being compared share 75% or greater, even more preferably 90% or greater of their nuclear genetic material. In another even more preferable aspect, a similar genetic background is a background where the organisms being compared are plants, and the plants are isogenic except for any genetic material originally introduced using plant transformation techniques.

In an embodiment of the present invention, a nucleic acid molecule, when introduced into a cell or organism, is capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD2* gene while leaving the level of a protein and/or transcript encoded by a second *FAD2* gene partially unaffected, substantially unaffected, or essentially unaffected. In a

preferred aspect, the capability of a nucleic acid molecule to selectively reduce the level of a gene relative to another gene is carried out by a comparison of levels of mRNA transcripts. As used herein, mRNA transcripts include processed and non-processed mRNA transcripts.

In another embodiment, a nucleic acid molecule, when introduced into a cell or organism, is capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD2-1* gene while leaving the level of a protein and/or transcript encoded by a *FAD2-2* gene partially unaffected, substantially unaffected, or essentially unaffected. In a different embodiment, a nucleic acid molecule, when introduced into a cell or organism, is capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD2-2* gene while leaving the level of a protein and/or transcript encoded by a *FAD2-1* gene partially unaffected, substantially unaffected, or essentially unaffected.

In a further embodiment, a nucleic acid molecule, when introduced into a cell or organism, is capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD2* gene while leaving the level of a protein and/or transcript encoded by a *FAD3* gene partially unaffected, substantially unaffected, or essentially unaffected. In a preferred embodiment, a nucleic acid molecule, when introduced into a cell or organism, is capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD2-1* gene while leaving the level of a protein and/or transcript encoded by a *FAD3* gene partially unaffected, substantially unaffected, or essentially unaffected.

In a different embodiment, a nucleic acid molecule, when introduced into a cell or organism, is capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD3* gene while leaving the level of a protein and/or transcript encoded by another *FAD3* gene partially unaffected, substantially unaffected, or essentially unaffected.

In an additional embodiment, a nucleic acid molecule, when introduced into a cell or organism, is capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD3-1C* gene while leaving the level of a protein and/or transcript encoded by a *FAD3-1B* gene partially unaffected, substantially unaffected, or essentially unaffected. In an additional embodiment, a nucleic acid molecule, when introduced into a cell or organism, is capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD3-1C* gene while

leaving the level of a protein and/or transcript encoded by a *FAD3-1A* gene partially unaffected, substantially unaffected, or essentially unaffected.

In a different embodiment, a nucleic acid molecule, when introduced into a cell or organism, is capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD3-1B* gene while leaving the level of a protein and/or transcript encoded by a *FAD3-1C* gene partially unaffected, substantially unaffected, or essentially unaffected. In a different embodiment, a nucleic acid molecule, when introduced into a cell or organism, is capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD3-1B* gene while leaving the level of a protein and/or transcript encoded by a *FAD3-1A* gene partially unaffected, substantially unaffected, or essentially unaffected.

In a further embodiment, a nucleic acid molecule, when introduced into a cell or organism, is capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD3-1A* gene while leaving the level of a protein and/or transcript encoded by a *FAD3-1B* gene partially unaffected, substantially unaffected, or essentially unaffected. In an additional embodiment, a nucleic acid molecule, when introduced into a cell or organism, is capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD3-1A* gene while leaving the level of a protein and/or transcript encoded by a *FAD3-1C* gene partially unaffected, substantially unaffected, or essentially unaffected.

Further preferred embodiments of the invention are nucleic acid molecules that are at least 50%, 60%, or 70% identical over their entire length to a nucleic acid molecule of the invention, and nucleic acid molecules that are complementary to such nucleic acid molecules. More preferable are nucleic acid molecules that comprise a region that is at least 80% or 85% identical over its entire length to a nucleic acid molecule of the invention and nucleic acid molecules that are complementary thereto. In this regard, nucleic acid molecules at least 90% identical over their entire length are particularly preferred, those at least 95% identical are especially preferred. Further, those with at least 97% identity are highly preferred and those with at least 98% and 99% identity are particularly highly preferred, with those at least 99% being the most highly preferred.

The invention also provides a nucleic acid molecule comprising a nucleic acid molecule sequence obtainable by screening an appropriate library containing the complete gene for a nucleic acid molecule sequence set forth in the Sequence Listing under stringent hybridization conditions with a probe having the sequence of said nucleic acid molecule sequence or a fragment thereof; and isolating said nucleic acid molecule sequence. Fragments useful for obtaining such a nucleic acid molecule include, for example, probes and primers as described herein.

Nucleic acid molecules of the invention can be used as a hybridization probe for RNA, cDNA, or genomic DNA to isolate full length cDNAs or genomic clones and to isolate cDNA or genomic clones of other genes that have a high sequence similarity to the sequence of a nucleic acid molecule set forth in the Sequence Listing.

The nucleic acid molecules of the present invention can be readily obtained by using the herein described nucleic acid molecules or fragments thereof to screen cDNA or genomic libraries obtained from plant species or other appropriate organisms. These methods are known to those of skill in the art, as are methods for forming such libraries. In one embodiment, such sequences are obtained by incubating nucleic acid molecules of the present invention with members of genomic libraries and recovering clones that hybridize to such nucleic acid molecules thereof. In a second embodiment, methods of chromosome walking or inverse PCR may be used to obtain such sequences. In a third embodiment, the sequence of a nucleic acid molecule of the present invention may be used to screen a library or database, using bioinformatics techniques known in the art. *See, e.g., Bioinformatics*, Baxevanis & Ouellette, eds., Wiley-Interscience (1998).

Any of a variety of methods may be used to obtain one or more of the nucleic acid molecules of the present invention. Automated nucleic acid synthesizers may be employed for this purpose, and to make a nucleic acid molecule that has a sequence also found in a cell or organism. In lieu of such synthesis, the disclosed nucleic acid molecules may be used to define a pair of primers that can be used with the polymerase chain reaction to amplify and obtain any desired nucleic acid molecule or fragment.

"Identity," as is well understood in the art, is a relationship between two or more polypeptide sequences or two or more nucleic acid molecule sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or nucleic acid molecule sequences, as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods including, but not limited to, those described in *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York (1988); *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part I*, Griffin, A.M. and Griffin, H.G., eds., Humana Press, New Jersey (1994); *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press (1987); *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., Stockton Press, New York (1991); and Carillo, H., and Lipman, D., SIAM *J. Applied Math*, 48:1073 (1988). Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available programs. Computer programs which can be used to determine identity between two sequences include, but are not limited to, GCG (Devereux, J., *et al.*, *Nucleic Acids Research* 12(1):387 (1984); suite of five BLAST programs, three designed for nucleotide sequences queries (BLASTN, BLASTX, and TBLASTX) and two designed for protein sequence queries (BLASTP and TBLASTN) (Coulson, *Trends in Biotechnology*, 12:76-80 (1994); Birren *et al.*, *Genome Analysis, 1*:543-559 (1997)). The BLASTX program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., *et al.*, NCBI NLM NIH, Bethesda, MD 20894; Altschul, S., *et al., J. Mol. Biol., 215*:403-410 (1990)). The well-known Smith Waterman algorithm can also be used to determine identity.

Parameters for polypeptide sequence comparison typically include the following:

Algorithm: Needleman and Wunsch, *J. Mol. Biol., 48*:443-453 (1970)

Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, *Proc. Natl. Acad. Sci. USA, 89*:10915-10919 (1992)

Gap Penalty: 12

Gap Length Penalty: 4

A program which can be used with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison, Wisconsin. The above parameters along with no penalty for end gap are the default parameters for peptide comparisons.

Parameters for nucleic acid molecule sequence comparison include the following:

Algorithm: Needleman and Wunsch, *J. Mol. Bio., 48*:443-453 (1970)

Comparison matrix: matches - +10; mismatches = 0

Gap Penalty: 50

Gap Length Penalty: 3

As used herein, "% identity" is determined using the above parameters as the default parameters for nucleic acid molecule sequence comparisons and the "gap" program from GCG, version 10.2.

The invention further relates to nucleic acid molecules that hybridize to nucleic acid molecules of the present invention. In particular, the invention relates to nucleic acid molecules that hybridize under stringent conditions to the above-described nucleic acid molecules. As used herein, the terms "stringent conditions" and "stringent hybridization conditions" mean that hybridization will generally occur if there is at least 95% and preferably at least 97% identity between the sequences. An example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising 50% formamide, 5x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ milliliter denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at approximately 65°C. Other hybridization and wash conditions are well known and are exemplified in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY (1989), particularly Chapter 11.

In embodiments where nucleic acid sequences which when expressed are capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD2-1* gene and the level of a protein and/or transcript encoded by at least one *FAD3* gene while leaving the level of a protein and/or transcript of a *FAD2-2* gene in the plant partially, substantially or essentially unaffected, preferred *FAD2-1* nucleic acid sequences are selected from the groups consisting of (1) nucleic acid sequences with at least 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or

100% sequence identity over the entire length of the nucleic acid molecule with a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and fragments thereof, where such a nucleic acid sequence does not hybridize under stringent conditions to a nucleic acid molecule with a nucleotide sequence of SEQ ID NO:4; (2) nucleic acid molecules which contain sequences that are also found in a soybean *FAD2-1* gene intron; and (3) nucleic acid molecules that exhibit sequences with at least 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or 100% sequence identity over the entire length of the nucleic acid molecule with a nucleic acid molecule of (2).

In embodiments where nucleic acid sequences which when expressed are capable of selectively reducing the level of a protein and/or transcript encoded by a *FAD2-2* gene and the level of a protein and/or transcript encoded by at least one *FAD3* gene while leaving the level of a protein and/or transcript of a *FAD2-1* gene in the plant partially, substantially or essentially unaffected, preferred *FAD2-2* nucleic acid sequences are selected from the groups consisting of (1) nucleic acid sequences with at least 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or 100% sequence identity over the entire length of the nucleic acid molecule with a nucleotide sequence of SEQ ID NO: 4 and fragments thereof, where such a nucleic acid sequence does not hybridize under stringent conditions to a nucleic acid molecule with a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2; (2) nucleic acid molecules which contain sequences that are also found in a soybean *FAD2-2* gene intron; and (3) nucleic acid molecules that exhibit sequences with at least 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or 100% sequence identity over the entire length of the nucleic acid molecule with a nucleic acid molecule of (2).

In embodiments where nucleic acid sequences which when expressed are capable of selectively reducing a *FAD3* gene, preferred *FAD3* nucleic acid sequences are selected from the groups consisting of (1) nucleic acid sequences with at least 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or 100% sequence identity over the entire length of the nucleic acid molecule with a nucleotide sequence selected from the group consisting of SEQ ID NOs:5-14, and fragments thereof, where such a nucleic acid sequence does not hybridize under stringent conditions to a nucleic acid molecule with a nucleotide sequence selected from the group

consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:4; (2) nucleic acid molecules which contain sequences that are also found in a soybean *FAD3* gene intron; and (3) nucleic acid molecules that exhibit sequences with at least 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or 100% sequence identity over the entire length of the nucleic acid molecule with a nucleic acid molecule of (2).

One subset of the nucleic acid molecules of the invention includes fragment nucleic acid molecules. Fragment nucleic acid molecules may consist of significant portion(s) of, or indeed most of, the nucleic acid molecules of the invention, such as those specifically disclosed. Alternatively, the fragments may comprise smaller oligonucleotides (having from about 15 to about 400 contiguous nucleotide residues and more preferably, about 15 to about 30 contiguous nucleotide residues, or about 50 to about 100 contiguous nucleotide residues, or about 100 to about 200 contiguous nucleotide residues, or about 200 to about 400 contiguous nucleotide residues, or about 275 to about 350 contiguous nucleotide residues).

In another aspect, a fragment nucleic acid molecule has a nucleic acid sequence that is at least 15, 25, 50, or 100 contiguous nucleotides of a nucleic acid molecule of the present invention. In a preferred embodiment, the nucleic acid molecule has a nucleic acid sequence that is at least 15, 25, 50, or 100 contiguous nucleotides of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:14 and complements thereof.

A fragment of one or more of the nucleic acid molecules of the present invention may be a probe and specifically a PCR probe. A PCR probe is a nucleic acid molecule capable of initiating a polymerase activity while in a double-stranded structure with another nucleic acid molecule. Various methods for determining the structure of PCR probes and PCR techniques exist in the art. Computer generated searches using programs such as Primer3 (www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi), STSPipeline (www-genome.wi.mit.edu/cgi-bin/www-STS_Pipeline), or GeneUp (Pesole *et al.*, *BioTechniques* *25*:112-123 (1998)), for example, can be used to identify potential PCR primers.

Nucleic acid molecules or fragments thereof of the present invention are capable of specifically hybridizing to other nucleic acid molecules under certain circumstances. Nucleic

acid molecules of the present invention include those that specifically hybridize to nucleic acid molecules having a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:14, complements thereof, and fragments of either.

As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure.

A nucleic acid molecule of the invention can also encode a homolog nucleic acid molecule. As used herein, a homolog nucleic acid molecule or fragment thereof is a counterpart nucleic acid molecule or fragment thereof in a second species (*e.g.*, corn *FAD2-1* intron nucleic acid molecule is a homolog of *Arabidopsis FAD2-1* intron nucleic acid molecule). A homolog can also be generated by molecular evolution or DNA shuffling techniques, so that the molecule retains at least one functional or structure characteristic of the original polypeptide (*see*, for example, U.S. Patent 5,811,238).

In another embodiment, the homolog is obtained from a plant selected from the group consisting of alfalfa, *Arabidopsis*, barley, *Brassica campestris*, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*, flax, an ornamental plant, jojoba, corn, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm. More particularly, a preferred homolog is obtained from a plant selected from the group consisting of canola, corn, *Brassica campestris*, oilseed rape, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, rapeseed, safflower, oil palm, flax, and sunflower. In an even more preferred embodiment, the homolog is obtained from a plant selected from the group consisting of canola, rapeseed, corn, *Brassica campestris*, oilseed rape, soybean, sunflower, safflower, oil palm, and peanut.

Plant Constructs and Plant Transformants

One or more of the nucleic acid molecules of the invention may be used in plant transformation or transfection. Exogenous genetic material may be transferred into a plant cell and the plant cell regenerated into a whole, fertile or sterile plant or plant part. Exogenous

genetic material is any genetic material, whether naturally occurring or otherwise, from any source that is capable of being inserted into any organism.

A plant can have a family of more than one *FAD2* or *FAD3* genes (*i.e.*, genes which encode an enzyme with the specified activity present at different locations within the genome of the plants). As used herein, a "*FAD2* gene family member" is any *FAD2* gene found within the genetic material of the plant. As used herein, a "*FAD3* gene family member" is any *FAD3* gene found within the genetic material of the plant. In one embodiment, a gene family can be additionally classified by the similarity of the nucleic acid sequences. In a preferred aspect of this embodiment, a gene family member exhibits at least 60%, more preferably at least 70%, more preferably at least 80% nucleic acid sequence identity in the coding sequence portion of the gene.

In one aspect of the present invention, a plant contains a double-stranded RNAi construct where a first promoter is operably linked to a first nucleic acid molecule, having a first nucleic acid sequence that has at least about 85% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:2, complements thereof, and fragments of either, and a second nucleic acid molecule that has a second nucleic acid sequence with at least about 85% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4 through SEQ ID NO:14, complements thereof, and fragments of either, is operably linked to the first nucleic acid molecule.

Also provided by the present invention is a plant comprising a double- stranded RNAi construct where a first promoter is operably linked to a first nucleic acid molecule, having a first nucleic acid sequence that has at least about 85% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:2, complements thereof, and fragments of either, wherein a second nucleic acid molecule having a second nucleic acid sequence that has at least about 85% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:4 through SEQ ID NO:14, complements thereof, and fragments of either is operably linked to a second promoter in a dsRNAi configuration.

In one embodiment of the invention, the expression level of a protein or transcript in one family member of that gene is selectively reduced while leaving the level of a protein or

transcript of a second family member partially unaffected. In a preferred embodiment of the invention, the expression level of a protein or transcript in one family member of that gene is selectively reduced while leaving the level of a protein or transcript of a second family member substantially unaffected. In a highly preferred embodiment of the invention, the expression level of a protein or transcript in one family member of that gene is selectively reduced while leaving the level of a protein or transcript of a second family member essentially unaffected.

In a particularly preferred embodiment, a plant of the present invention includes nucleic acid sequences which when expressed are capable of selectively reducing the expression level of a protein and/or transcript encoded by certain *FAD2* and *FAD3* genes while leaving the level of a protein and/or transcript of at least one other *FAD2* or *FAD3* gene in the plant partially unaffected. In a particularly preferred embodiment, a plant of the present invention includes nucleic acid sequences which when expressed are capable of selectively reducing the expression level of a protein and/or transcript encoded by certain *FAD2* and *FAD3* genes while leaving the level of a protein and/or transcript of at least one other *FAD2* or *FAD3* gene in the plant substantially unaffected. In a particularly preferred embodiment, a plant of the present invention includes nucleic acid sequences which when expressed are capable of selectively reducing the expression level of a protein and/or transcript encoded by certain *FAD2* and *FAD3* genes while leaving the level of a protein and/or transcript of at least one other *FAD2* or *FAD3* gene in the plant essentially unaffected.

In a more particularly preferred embodiment, a soybean plant of the present invention includes nucleic acid sequences which when expressed are capable of selectively reducing the expression level of a protein and/or transcript encoded by a *FAD2-1* gene and at least one *FAD3* gene while leaving the level of a protein and/or transcript of a *FAD2-2* gene in the plant partially unaffected. In a more particularly preferred embodiment, a soybean plant of the present invention includes nucleic acid sequences which when expressed are capable of selectively reducing the expression level of a protein and/or transcript encoded by a *FAD2-1* gene and at least one *FAD3* gene while leaving the level of a protein and/or transcript of a *FAD2-2* gene in the plant substantially unaffected. In a more particularly preferred embodiment, a soybean plant of the present invention includes nucleic acid sequences which when expressed are capable of

selectively reducing the expression level of a protein and/or transcript encoded by a *FAD2-1* gene and at least one *FAD3* gene while leaving the level of a protein and/or transcript of a *FAD2-2* gene in the plant essentially unaffected.

In another more particularly preferred embodiment, a soybean plant of the present invention includes nucleic acid sequences which when expressed are capable of selectively reducing the expression level of a protein and/or transcript encoded by a *FAD2-1* gene and at least two, three or more *FAD3* genes while leaving the level of a protein and/or transcript of a *FAD2-2* gene in the plant partially unaffected. In another more particularly preferred embodiment, a soybean plant of the present invention includes nucleic acid sequences which when expressed are capable of selectively reducing the expression level of a protein and/or transcript encoded by a *FAD2-1* gene and at least two, three or more *FAD3* genes while leaving the level of a protein and/or transcript of a *FAD2-2* gene in the plant substantially unaffected. In another more particularly preferred embodiment, a soybean plant of the present invention includes nucleic acid sequences which when expressed are capable of selectively reducing the expression level of a protein and/or transcript encoded by a *FAD2-1* gene and at least two, three or more *FAD3* genes while leaving the level of a protein and/or transcript of a *FAD2-2* gene in the plant essentially unaffected.

In a preferred embodiment, a soybean of the present invention includes exogenous nucleic acid sequences selected from the groups consisting of a *FAD3* intron or fragment thereof, more preferably from a nucleic acid molecule selected from the group consisting of SEQ ID NOs:5-14, or fragments thereof.

In a particularly preferred embodiment, a soybean of the present invention includes a nucleic acid sequence which when expressed is capable of reducing the expression level of a protein and/or transcript encoded by a *FAD3-1C* gene while leaving the level of a protein and/or transcript of a *FAD3-1B* gene in the plant partially unaffected. In a particularly preferred embodiment, a soybean of the present invention includes a nucleic acid sequence which when expressed is capable of reducing the expression level of a protein and/or transcript encoded by a *FAD3-1C* gene while leaving the level of a protein and/or transcript of a *FAD3-1B* gene in the plant substantially unaffected. In a particularly preferred embodiment, a soybean of the present

invention includes a nucleic acid sequence which when expressed is capable of reducing the expression level of a protein and/or transcript encoded by a *FAD3-1C* gene while leaving the level of a protein and/or transcript of a *FAD3-1B* gene in the plant essentially unaffected.

In embodiments where nucleic acid sequences which when expressed are capable of selectively reducing the expression level of a protein and/or transcript encoded by a *FAD2-1* gene and at least one *FAD3* genes while leaving the level of a protein and/or transcript of a *FAD2-2* gene in the plant partially unaffected, substantially unaffected, or essentially unaffected, preferred *FAD2-1* nucleic acid sequences are selected from the groups consisting of (1) nucleic acid sequences with at least 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or 100% sequence identity over the entire length of the nucleic acid molecule with a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and fragments thereof, where such a nucleic acid sequence does not hybridize under stringent conditions to a nucleic acid molecule with a nucleotide sequence of SEQ ID NO:4; (2) nucleic acid molecules which contain sequences that are also found in a soybean *FAD2-1* gene intron; and (3) nucleic acid molecules that exhibit sequences with at least 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or 100% sequence identity over the entire length of the nucleic acid molecule with a nucleic acid molecule of (2).

In embodiments where nucleic acid sequences which when expressed are capable of selectively reducing the expression level of a protein and/or transcript encoded by a *FAD3* gene, preferred *FAD3* nucleic acid sequences are selected from the groups consisting of (1) nucleic acid sequences with at least 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or 100% sequence identity over the entire length of the nucleic acid molecule with a nucleotide sequence selected from the group consisting of SEQ ID NOs:5-14, and fragments thereof, where such a nucleic acid sequence does not hybridize under stringent conditions to a nucleic acid molecule with a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:4; (2) nucleic acid molecules which contain sequences that are also found in a soybean *FAD3* gene intron; and (3) nucleic acid molecules that exhibit sequences with at least 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or 100% sequence identity over the entire length of the nucleic acid molecule with a nucleic acid molecule of (2).

In a preferred embodiment, a soybean seed of the present invention has an oil composition that is 50% or greater oleic acid and 10% or less linolenic acid. In a more preferred embodiment, a soybean seed of the present invention has an oil composition that is 60% or greater oleic acid and 7% or less linolenic acid. In a particular preferred embodiment, a soybean seed of the present invention has an oil composition that is 65% or greater oleic acid and 5% or less linolenic acid, preferably 4% or less linolenic acid, and more preferably 3% or less linolenic acid. As used herein, all % composition of oils within a plant or plant part such as a seed are determined by relative mole percent.

In another preferred embodiment a soybean seed of the present invention has an oil composition that is between 50% and 90% oleic acid, and 10% or less linolenic acid. In a more preferred embodiment, a soybean seed of the present invention has an oil composition that is between 60% and 80% oleic acid, and 7% or less linolenic acid. In a particular preferred embodiment, a soybean seed of the present invention has an oil composition that is between 65% and 75% oleic acid, and 5% or less linolenic acid, preferably 4% or less linolenic acid, and more preferably 3% or less linolenic acid.

In another preferred embodiment a soybean seed of the present invention has an oil composition that is between 50% and 90% oleic acid, between 8% and 16% palmitic acid and 10% or less linolenic acid. In a more preferred embodiment, a soybean seed of the present invention has an oil composition that is between 60% and 80% oleic acid, between 6% and 12% palmitic acid and 7% or less linolenic acid. In a particularly preferred embodiment, a soybean seed of the present invention has an oil composition that is between 65% and 75% oleic acid, between 8% and 11% palmitic acid and 5% or less linolenic acid, preferably 4% or less linolenic acid, and more preferably 3% or less linolenic acid.

In a particularly preferred embodiment, a soybean seed of the present invention has an oil composition that is between 65% and 75% oleic acid, and 5% or less linolenic acid, preferably 4% or less linolenic acid, and more preferably 3% or less linolenic acid, where nucleic acid sequences which when expressed are capable of selectively reducing the expression level of a protein and/or transcript encoded by a *FAD2-1* gene and at least one *FAD3* gene while leaving the level of a protein and/or transcript of a *FAD2-2* gene in the plant partially unaffected,

substantially unaffected, or essentially unaffected, the *FAD2-1* nucleic acid sequences are selected from the groups consisting of: (1) nucleic acid sequences with at least 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or 100% sequence identity over the entire length of the nucleic acid molecule with a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and fragments thereof, where such a nucleic acid sequence does not hybridize under stringent conditions to a nucleic acid molecule with a nucleotide sequence of SEQ ID NO:4; (2) nucleic acid molecules which contain sequences that are also found in a soybean *FAD2-1* gene intron; and (3) nucleic acid molecules that exhibit sequences with at least 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or 100% sequence identity over the entire length of the nucleic acid molecule with a nucleic acid molecule of (2); and the *FAD3* nucleic acid sequences are selected from the groups consisting of (1) nucleic acid sequences with at least 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or 100% sequence identity over the entire length of the nucleic acid molecule with a nucleotide sequence selected from the group consisting of SEQ ID NOs:5-14, and fragments thereof, where such a nucleic acid sequence does not hybridize under stringent conditions to a nucleic acid molecule with a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:4; (2) nucleic acid molecules which contain sequences that are also found in a soybean *FAD3* gene intron; and (3) nucleic acid molecules that exhibit sequences with at least 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or 100% sequence identity over the entire length of the nucleic acid molecule with a nucleic acid molecule of (2).

In another embodiment, a soybean seed of the present invention has an oil composition that is 80% or greater, more preferably 90% or greater oleic acid and 5% or less linolenic acid, preferably 4% or less linolenic acid, and more preferably 3% or less linolenic acid.

In a preferred embodiment, a soybean seed of the present invention has an oil composition that is 80% or greater, more preferably 90% or greater oleic acid and 5% or less linolenic acid, preferably 4% or less linolenic acid, and more preferably 3% or less linolenic acid, where the nucleic acid sequences are capable of reducing the expression of *FAD2-1*, *FAD2-2* and at least one *FAD3* genes. In a particularly preferred embodiment of this aspect, the

nucleic acid sequences are selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:14, complements thereof, and fragments of either.

In a preferred embodiment of the present invention, a soybean seed of the present invention has an oil composition of 50% or greater oleic acid, more preferably 60% or greater, 70% or greater, 80% or greater, or 90% or greater oleic acid.

In another preferred embodiment of the present invention, a soybean seed of the present invention has an oil composition that is 10% or less linolenic acid, more preferably 5% or less, 4% or less, or 3% or less linolenic acid.

Analogous genetic material may also be obtained from other species, for example monocotyledons or dicotyledons, including, but not limited to canola, corn, soybean, *Arabidopsis, Phaseolus,* peanut, alfalfa, wheat, rice, oat, sorghum, rapeseed, rye, barley, millet, fescue, perennial ryegrass, sugarcane, cranberry, papaya, banana, safflower, oil palm, flax, muskmelon, apple, cucumber, dendrobium, gladiolus, chrysanthemum, liliacea, cotton, eucalyptus, sunflower, *Brassica campestris,* oilseed rape, turfgrass, sugarbeet, coffee and dioscorea (Christou, INO: *Particle Bombardment for Genetic Engineering of Plants,* Biotechnology Intelligence Unit. Academic Press, San Diego, California (1996)), with canola, corn, *Brassica campestris,* oilseed rape, rapeseed, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax, and sunflower preferred, and canola, rapeseed, corn, *Brassica campestris,* oilseed rape, soybean, sunflower, safflower, oil palms, and peanut more preferred. In a more preferred embodiment, the genetic material is transferred into canola. In another more preferred embodiment, the genetic material is transferred into oilseed rape. In another particularly preferred embodiment, the genetic material is transferred into soybean.

The levels of products such as transcripts or proteins may be increased or decreased throughout an organism such as a plant or localized in one or more specific organs or tissues of the organism. For example the levels of products may be increased or decreased in one or more of the tissues and organs of a plant including without limitation: roots, tubers, stems, leaves, stalks, fruit, berries, nuts, bark, pods, seeds and flowers. A preferred organ is a seed.

Exogenous genetic material may be transferred into a host cell by the use of a DNA vector or construct designed for such a purpose. Design of such a vector is generally within the skill of the art (*See, Plant Molecular Biology: A Laboratory Manual*, Clark (ed.), Springer, New York (1997)).

A construct or vector may include a plant promoter to express the nucleic acid molecule of choice. In a preferred embodiment, any nucleic acid molecules described herein can be operably linked to a promoter region which functions in a plant cell to cause the production of an mRNA molecule. For example, any promoter that functions in a plant cell to cause the production of an mRNA molecule, such as those promoters described herein, without limitation, can be used. In a preferred embodiment, the promoter is a plant promoter.

A number of promoters that are active in plant cells have been described in the literature. These include, but are not limited to, the nopaline synthase (NOS) promoter (Ebert *et al., Proc. Natl. Acad. Sci. (U.S.A.) 84*:5745-5749 (1987)), the octopine synthase (OCS) promoter (which is carried on tumor-inducing plasmids of *Agrobacterium tumefaciens*), the caulimovirus promoters such as the cauliflower mosaic virus (CaMV) 19S promoter (Lawton *et al., Plant Mol. Biol. 9*:315-324 (1987)) and the CaMV 35S promoter (Odell *et al., Nature 313*:810-812 (1985)), the figwort mosaic virus 35S-promoter (U.S. Patent No. 5,378,619), the light-inducible promoter from the small subunit of ribulose-1,5-bis-phosphate carboxylase (ssRUBISCO), the Adh promoter (Walker *et al., Proc. Natl. Acad. Sci. (U.S.A.) 84*:6624-6628 (1987)), the sucrose synthase promoter (Yang *et al., Proc. Natl. Acad. Sci. (U.S.A.) 87*:4144-4148 (1990)), the R gene complex promoter (Chandler *et al., The Plant Cell 1*:1175-1183 (1989)) and the chlorophyll a/b binding protein gene promoter. These promoters have been used to create DNA constructs that have been expressed in plants; *see, e.g.*, PCT publication WO 84/02913. The CaMV 35S promoters are preferred for use in plants. Promoters known or found to cause transcription of DNA in plant cells can be used in the invention.

Particularly preferred promoters can also be used to express a nucleic acid molecule of the present invention in seeds or fruits. Indeed, in a preferred embodiment, the promoter used is a seed specific promoter. Examples of such promoters include the 5' regulatory regions from such genes as napin (Kridl *et al., Seed Sci. Res.* 1:209:219 (1991)), phaseolin (Bustos, *et al.,*

Plant Cell, 1(9):839-853 (1989)), soybean trypsin inhibitor (Riggs, *et al., Plant Cell* 1(6):609-621 (1989)), ACP (Baerson, *et al., Plant Mol. Biol.*, 22(2):255-267 (1993)), stearoyl-ACP desaturase (Slocombe, *et al., Plant Physiol.* 104(4):167-176 (1994)), soybean a' subunit of b-conglycinin (soy 7s, (Chen *et al., Proc. Natl. Acad. Sci.*, 83:8560-8564 (1986))), and oleosin (see, for example, Hong, *et al., Plant Mol. Biol.*, 34(3):549-555 (1997)). Further examples include the promoter for β-conglycinin (Chen *et al., Dev. Genet. 10*: 112-122 (1989)) and the promoter for FAE (PCT Publication WO 01/11061). Preferred promoters for expression in the seed are 7S and napin promoters.

Additional promoters that may be utilized are described, for example, in U.S. Patents 5,378,619; 5,391,725; 5,428,147; 5,447,858; 5,608,144; 5,608,144; 5,614,399; 5,633,441; 5,633,435; and 4,633,436. In addition, a tissue specific enhancer may be used (Fromm *et al., The Plant Cell 1*:977-984 (1989)).

Constructs or vectors may also include, with the region of interest, a nucleic acid sequence that acts, in whole or in part, to terminate transcription of that region. A number of such sequences have been isolated, including the Tr7 3' sequence and the NOS 3' sequence (Ingelbrecht *et al., The Plant Cell 1*:671-680 (1989); Bevan *et al., Nucleic Acids Res. 11*:369-385 (1983)). Regulatory transcript termination regions can be provided in plant expression constructs of this invention as well. Transcript termination regions can be provided by the DNA sequence encoding the gene of interest or a convenient transcription termination region derived from a different gene source, for example, the transcript termination region that is naturally associated with the transcript initiation region. The skilled artisan will recognize that any convenient transcript termination region that is capable of terminating transcription in a plant cell can be employed in the constructs of the present invention.

A vector or construct may also include regulatory elements. Examples of such include the Adh intron 1 (Callis *et al., Genes and Develop. 1*:1183-1200 (1987)), the sucrose synthase intron (Vasil *et al., Plant Physiol. 91*:1575-1579 (1989)) and the TMV omega element (Gallie *et al., The Plant Cell 1*:301-311 (1989)). These and other regulatory elements may be included when appropriate.

A vector or construct may also include a selectable marker. Selectable markers may also be used to select for plants or plant cells that contain the exogenous genetic material. Examples of such include, but are not limited to: a *neo* gene (Potrykus *et al., Mol. Gen. Genet. 199*:183-188 (1985)), which codes for kanamycin resistance and can be selected for using kanamycin, RptII, G418, hpt; a bar gene which codes for bialaphos resistance; a mutant EPSP synthase gene (Hinchee *et al., Bio/Technology 6*:915-922 (1988); Reynaerts *et al.,* Selectable and Screenable Markers. In Gelvin and Schilperoort. Plant Molecular Biology Manual, Kluwer, Dordrecht (1988); Reynaerts *et al.,* Selectable and screenable markers. In Gelvin and Schilperoort. Plant Molecular Biology Manual, Kluwer, Dordrecht (1988)), *aad*A (Jones *et al., Mol. Gen. Genet.* (1987)),) which encodes glyphosate resistance; a nitrilase gene which confers resistance to bromoxynil (Stalker *et al., J. Biol. Chem. 263*:6310-6314 (1988)); a mutant acetolactate synthase gene (ALS) which confers imidazolinone or sulphonylurea resistance (European Patent Application 154,204 (Sept. 11, 1985)), ALS (D'Halluin *et al.,* Bio/Technology 10: 309-314 (1992)), and a methotrexate resistant DHFR gene (Thillet *et al., J. Biol. Chem. 263*:12500-12508 (1988)).

A vector or construct may also include a screenable marker. Screenable markers may be used to monitor expression. Exemplary screenable markers include: a β-glucuronidase or *uid*A gene (GUS) which encodes an enzyme for which various chromogenic substrates are known (Jefferson, *Plant Mol. Biol, Rep. 5:*387-405 (1987); Jefferson *et al., EMBO J. 6:*3901-3907 (1987)); an R-locus gene, which encodes a product that regulates the production of anthocyanin pigments (red color) in plant tissues (Dellaporta *et al.*, Stadler Symposium *11*:263-282 (1988)); a β-lactamase gene (Sutcliffe *et al., Proc. Natl. Acad. Sci. (U.S.A.) 75*:3737-3741 (1978)), a gene which encodes an enzyme for which various chromogenic substrates are known (*e.g.*, PADAC, a chromogenic cephalosporin); a luciferase gene (Ow *et al., Science 234*:856-859 (1986)); a *xyl*E gene (Zukowsky *et al., Proc. Natl. Acad. Sci. (U.S.A.) 80*:1101-1105 (1983)) which encodes a catechol dioxygenase that can convert chromogenic catechols; an α-amylase gene (Ikatu *et al., Bio/Technol. 8*:241-242 (1990)); a tyrosinase gene (Katz *et al., J. Gen. Microbiol. 129*:2703-2714 (1983)) which encodes an enzyme capable of oxidizing tyrosine to DOPA and

dopaquinone which in turn condenses to melanin; an α-galactosidase, which will turn a chromogenic α-galactose substrate.

Included within the terms "selectable or screenable marker genes" are also genes that encode a secretable marker whose secretion can be detected as a means of identifying or selecting for transformed cells. Examples include markers that encode a secretable antigen that can be identified by antibody interaction, or even secretable enzymes that can be detected catalytically. Secretable proteins fall into a number of classes, including small, diffusible proteins that are detectable, (*e.g.*, by ELISA), small active enzymes that are detectable in extracellular solution (*e.g.*, α-amylase, β-lactamase, phosphinothricin transferase), or proteins that are inserted or trapped in the cell wall (such as proteins that include a leader sequence such as that found in the expression unit of extension or tobacco PR-S). Other possible selectable and/or screenable marker genes will be apparent to those of skill in the art.

It is understood that two or more nucleic molecules of the present invention may be introduced into a plant using a single construct and that construct can contain more than one promoter. In embodiments where the construct is designed to express two nucleic acid molecules, it is preferred that the two promoters are (i) two constitutive promoters, (ii) two seed-specific promoters, or (iii) one constitutive promoter and one seed-specific promoter. Preferred seed-specific and constitutive promoters are a napin and a CaMV promoter, respectively. Illustrative combinations are set forth in Example 8. It is understood that two or more of the nucleic molecules may be physically linked and expressed utilizing a single promoter, preferably a seed-specific or constitutive promoter.

It is further understood that two or more nucleic acids of the present invention may be introduced into a plant using two or more different constructs. Alternatively, two or more nucleic acids of the present invention may be introduced into two different plants and the plants may be crossed to generate a single plant expressing two or more nucleic acids. In an RNAi embodiment, it is understood that the sense and antisense strands may be introduced into the same plant on one construct or two constructs. Alternatively, the sense and antisense strands may be introduced into two different plants and the plants may be crossed to generate a single plant expressing both sense and antisense strands.

Any of the nucleic acid molecules and constructs of the invention may be introduced into a plant or plant cell in a permanent or transient manner. Preferred nucleic acid molecules and constructs of the present invention are described above in the Detailed Description, and in the Examples. Another embodiment of the invention is directed to a method of producing transgenic plants which generally comprises the steps of selecting a suitable plant or plant cell, transforming the plant or plant cell with a recombinant vector, and obtaining a transformed host cell.

In a preferred embodiment the plant or cell is, or is derived from, a plant involved in the production of vegetable oils for edible and industrial uses. Especially preferred are temperate oilseed crops. Plants of interest include, but are not limited to, rapeseed (canola and High Erucic Acid varieties), maize, soybean, crambe, mustard, castor bean, peanut, sesame, cotton, linseed, safflower, oil palm, flax, sunflower, and coconut. The invention is applicable to monocotyledonous or dicotyledonous species alike, and will be readily applicable to new and/or improved transformation and regulatory techniques.

Methods and technology for introduction of DNA into plant cells are well known to those of skill in the art, and virtually any method by which nucleic acid molecules may be introduced into a cell is suitable for use in the present invention. Non-limiting examples of suitable methods include: chemical methods; physical methods such as microinjection, electroporation, the gene gun, microprojectile bombardment, and vacuum infiltration; viral vectors; and receptor-mediated mechanisms. Other methods of cell transformation can also be used and include but are not limited to introduction of DNA into plants by direct DNA transfer into pollen, by direct injection of DNA into reproductive organs of a plant, or by direct injection of DNA into the cells of immature embryos followed by the rehydration of desiccated embryos.

Agrobacterium-mediated transfer is a widely applicable system for introducing genes into plant cells. *See, e.g.*, Fraley *et al., Bio/Technology 3*:629-635 (1985); Rogers *et al., Methods Enzymol. 153*:253-277 (1987). The region of DNA to be transferred is defined by the border sequences and intervening DNA is usually inserted into the plant genome. Spielmann *et al., Mol. Gen. Genet. 205*:34 (1986). Modern *Agrobacterium* transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium*, allowing for convenient manipulations. Klee

et al., In: *Plant DNA Infectious Agents*, Hohn and Schell (eds.), Springer-Verlag, New York, pp. 179-203 (1985).

The regeneration, development and cultivation of plants from single plant protoplast transformants or from various transformed explants is well known in the art. *See generally*, Maliga *et al.*, *Methods in Plant Molecular Biology,* Cold Spring Harbor Press (1995); Weissbach and Weissbach, In: *Methods for Plant Molecular Biology*, Academic Press, San Diego, CA (1988). Plants of the present invention can be part of or generated from a breeding program, and may also be reproduced using apomixis. Methods for the production of apomictic plants are known in the art. *See, e.g.*, U.S. Patent 5,811,636.

Cosuppression is the reduction in expression levels, usually at the level of RNA, of a particular endogenous gene or gene family by the expression of a homologous sense construct that is capable of transcribing mRNA of the same strandedness as the transcript of the endogenous gene (Napoli *et al.*, *Plant Cell 2*:279-289 (1990); van der Krol *et al.*, *Plant Cell 2*:291-299 (1990)). Cosuppression may result from stable transformation with a single copy nucleic acid molecule that is homologous to a nucleic acid sequence found with the cell (Prolls and Meyer, *Plant J. 2*:465-475 (1992)) or with multiple copies of a nucleic acid molecule that is homologous to a nucleic acid sequence found with the cell (Mittlesten *et al.*, *Mol. Gen. Genet. 244*:325-330 (1994)). Genes, even though different, linked to homologous promoters may result in the cosuppression of the linked genes (Vaucheret, *C.R. Acad. Sci. III 316*:1471-1483 (1993); Flavell, *Proc. Natl. Acad. Sci. (U.S.A.) 91*:3490-3496 (1994)); van Blokland *et al.*, *Plant J. 6*:861-877 (1994); Jorgensen, *Trends Biotechnol. 8*:340-344 (1990); Meins and Kunz, In: *Gene Inactivation and Homologous Recombination in Plants*, Paszkowski (ed.), pp. 335-348, Kluwer Academic, Netherlands (1994))(Kinney, Induced Mutations and Molecular Techniques for Crop Improvement, Proceedings of a Symposium 19-23 June 1995 (jointly organized by IAEA and FA)), pages 101 –113 (IAEA-SM 340-49).

It is understood that one or more of the nucleic acids of the invention may be introduced into a plant cell and transcribed using an appropriate promoter with such transcription resulting in the cosuppression of an endogenous protein. Such nucleic acid molecules may be operably linked to the same promoter in polycistronic configuration or to different promoters.

Antisense approaches are a way of preventing or reducing gene function by targeting the genetic material (Mol *et al., FEBS Lett. 268*:427-430 (1990)). The objective of the antisense approach is to use a sequence complementary to the target gene to block its expression and create a mutant cell line or organism in which the level of a single chosen protein is selectively reduced or abolished. Antisense techniques have several advantages over other 'reverse genetic' approaches. The site of inactivation and its developmental effect can be manipulated by the choice of promoter for antisense genes or by the timing of external application or microinjection. Antisense can manipulate its specificity by selecting either unique regions of the target gene or regions where it shares homology to other related genes (Hiatt *et al.*, In: *Genetic Engineering*, Setlow (ed.), Vol. 11, New York: Plenum 49-63 (1989)).

Antisense RNA techniques involve introduction of RNA that is complementary to the target mRNA into cells, which results in specific RNA:RNA duplexes being formed by base pairing between the antisense substrate and the target mRNA (Green *et al., Annu. Rev. Biochem. 55*:569-597 (1986)). Under one embodiment, the process involves the introduction and expression of an antisense gene sequence. Such a sequence is one in which part or all of the normal gene sequences are placed under a promoter in inverted orientation so that the 'wrong' or complementary strand is transcribed into a noncoding antisense RNA that hybridizes with the target mRNA and interferes with its expression (Takayama and Inouye, *Crit. Rev. Biochem. Mol. Biol. 25*:155-184 (1990)). An antisense vector is constructed by standard procedures and introduced into cells by methods including but not limited to transformation, transfection, electroporation, microinjection, and infection. The type of transformation and choice of vector will determine whether expression is transient or stable. The promoter used for the antisense gene may influence the level, timing, tissue, specificity, or inducibility of the antisense inhibition.

It has been reported that the introduction of double-stranded RNA into cells may also be used to disrupt the function of an endogenous gene. (Fire *et al., Nature 391*: 806-811 (1998)). Such disruption has been demonstrated, for example, in *Caenorhabditis elegans* and is often referred to as RNA interference, or RNAi. (Fire *et al., Nature 391*: 806-811 (1998)). The disruption of gene expression in *C. elegans* by double-stranded RNA has been reported to

induce suppression by a post-transcriptional mechanism. (Montgomery *et al.*, Proc. Natl. Acad. Sci. 95:15502-15507 (1998)). Evidence of gene silencing by double-stranded RNA has also been reported for plants. (Waterhouse *et al., Proc. Natl. Acad. Sci. 95*: 13959-13964 (1998)).

An intron-spliced hairpin structure reportedly may also be used to effect post-transcriptional gene suppression. (Smith *et al., Nature 407*: 319-320 (2000)). Reports indicate that post-transcriptional gene silencing can be induced with almost 100% efficiency by the use of intron-spliced RNA with a hairpin structure. (Smith *et al., Nature 407*: 319-320 (2000)).

It is understood that one or more of the nucleic acids of the invention may be modified in order to effect RNAi or another mode of post-transcriptional gene suppression.

The present invention also provides for parts of the plants, particularly reproductive or storage parts. Plant parts, without limitation, include seed, endosperm, ovule, pollen, roots, tubers, stems, leaves, stalks, fruit, berries, nuts, bark, pods, seeds and flowers. In a particularly preferred embodiment of the present invention, the plant part is a seed.

The present invention also provides a container of over 10,000, more preferably 20,000, and even more preferably 40,000 seeds where over 10%, more preferably 25%, more preferably 50% and even more preferably 75% or 90% of the seeds are seeds derived from a plant of the present invention.

The present invention also provides a container of over 10 kg, more preferably 25 kg, and even more preferably 50 kg seeds where over 10%, more preferably 25%, more preferably 50% and even more preferably 75% or 90% of the seeds are seeds derived from a plant of the present invention.

Any of the plants or parts thereof of the present invention may be processed to produce a feed, meal, protein, or oil preparation. A particularly preferred plant part for this purpose is a seed. In a preferred embodiment the feed, meal, protein or oil preparation is designed for livestock animals or humans, or both. Methods to produce feed, meal, protein and oil preparations are known in the art. See, for example, U.S. Patents 4,957,748, 5,100,679, 5,219,596, 5,936,069, 6,005,076, 6,146,669, and 6,156,227. In a preferred embodiment, the protein preparation is a high protein preparation. Such a high protein preparation preferably has a protein content of greater than 5% w/v, more preferably 10% w/v, and even more preferably

15% w/v. In a preferred oil preparation, the oil preparation is a high oil preparation with an oil content derived from a plant or part thereof of the present invention of greater than 5% w/v, more preferably 10% w/v, and even more preferably 15% w/v. In a preferred embodiment the oil preparation is a liquid and of a volume greater than 1, 5, 10 or 50 liters. The present invention provides for oil produced from plants of the present invention or generated by a method of the present invention. Such an oil may exhibit enhanced oxidative stability. Also, such oil may be a minor or major component of any resultant product. Moreover, such oil may be blended with other oils. In a preferred embodiment, the oil produced from plants of the present invention or generated by a method of the present invention constitutes greater than 0.5%, 1%, 5%, 10%, 25%, 50%, 75% or 90% by volume or weight of the oil component of any product. In another embodiment, the oil preparation may be blended and can constitute greater than 10%, 25%, 35%, 50% or 75% of the blend by volume. Oil produced from a plant of the present invention can be admixed with one or more organic solvents or petroleum distillates.

In one embodiment, an oil of the present invention has an oil composition that is 50% or greater oleic acid and 10% or less linolenic acid. In another embodiment, an oil of the present invention has an oil composition that is 60% or greater oleic acid and 7% or less linolenic acid. In another embodiment, an oil of the present invention has an oil composition that is 65% or greater oleic acid and 5% or less linolenic acid, preferably 4% or less linolenic acid, and more preferably 3% or less linolenic acid.

In another embodiment, an oil of the present invention has an oil composition that is between 50% and 90% oleic acid and 10% or less linolenic acid. In another embodiment, an oil of the present invention has an oil composition that is between 60% and 80% oleic acid and 7% or less linolenic acid. In another embodiment, an oil of the present invention has an oil composition that is between 65% and 75% oleic acid and 5% or less linolenic acid, preferably 4% or less linolenic acid, and more preferably 3% or less linolenic acid.

In another embodiment, an oil of the present invention has an oil composition that is 80% or greater, more preferably 90% or greater oleic acid and 5% or less linolenic acid, preferably 4% or less linolenic acid, and more preferably 3% or less linolenic acid. In another embodiment, an oil of the present invention has an oil composition that is 50% or greater oleic

acid, more preferably 60% or greater, 70% or greater, 80% or greater, or 90% or greater oleic acid. In another embodiment, an oil of the present invention has an oil composition that is 10% or less linolenic acid, preferably 5% or less, 4% or less, or 3% or less linolenic acid.

Plants of the present invention can be part of or generated from a breeding program. The choice of breeding method depends on the mode of plant reproduction, the heritability of the trait(s) being improved, and the type of cultivar used commercially (*e.g.*, F_1 hybrid cultivar, pureline cultivar, etc). Selected, non-limiting approaches, for breeding the plants of the present invention are set forth below. A breeding program can be enhanced using marker assisted selection of the progeny of any cross. It is further understood that any commercial and non-commercial cultivars can be utilized in a breeding program. Plants of the present invention can be part of or generated from a breeding program. The choice of breeding method depends on the mode of plant reproduction, the heritability of the trait(s) being improved, and the type of cultivar used commercially (*e.g.*, F_1 hybrid cultivar, pureline cultivar, etc). Selected, non-limiting approaches, for breeding the plants of the present invention are set forth below. A breeding program can be enhanced using marker assisted selection of the progeny of any cross. It is further understood that any commercial and non-commercial cultivars can be utilized in a breeding program.

In a non-limiting example of breeding, a soybean *FAD2-1A* intron suppressed line can be used to pollinate a low linolenic soybean *FAD3* mutant line derived from a spontaneous mutation, or derived from but not limited to methods such as Targeted Induced Local Lesions in Genomes (TILLING) (McCallum et al., Nature Biotech, 18:455-457 (2000)), gene replacement via RNA/DNA chimeric oligonucleotides, homologous recombination, T-DNA or transposan mutagenesis. RNA from soybean seeds containing both one or more expressed *FAD2* intron regions and *FAD3* mutations, including knock out, can be screened using Northern blots (as described in Example 5) to determine the levels of *FAD2-1*, *FAD2-2*, *FAD3-1A*, *FAD3-1C* and *FAD3-1B* transcripts. The resulting plant is expected to have the attributes of both parents. Soybean plants with undetectable or low levels of *FAD2* or *FAD3* transcripts can be screened for fatty acid composition.

Plants of the present invention can be part of or generated from a breeding program, for example, where a soybean *FAD3-1A, FAD3-1B, and/or FAD3-1 C* intron suppressed line is used to pollinate soybean plants with elevated levels of oleic acid containing a *FAD2* mutant line derived from a spontaneous mutation, or derived from non-limiting methods such as, for example, TILLING, gene replacement via RNA/DNA chimeric oligonucleotides, homologous recombination, T-DNA or transposan mutagenesis. RNA from soybean seeds containing both one or more expressed *FAD3* intron regions and *FAD2* mutations, including knock out, can be screened using Northern blotting (as described in Example 5) to determine the levels of *FAD2-1*, *FAD2-2*, *FAD3-1A*, *FAD3-1B*, and *FAD3-1C* transcripts. Soybean plants with undetectable or low levels of *FAD2* or *FAD3* transcripts can be screened for fatty acid composition. Resulting plants are expected to have the attributes of both parents.

The development of new cultivars requires the development and selection of varieties, the crossing of these varieties and the selection of superior hybrid crosses. The hybrid seed can be produced by manual crosses between selected male-fertile parents or by using male sterility systems. Hybrids are selected for certain single gene traits such as pod color, flower color, seed yield, pubescence color, or herbicide resistance, which indicate that the seed is truly a hybrid. Additional data on parental lines, as well as the phenotype of the hybrid, influence the breeder's decision whether to continue with the specific hybrid cross. The parents in such hybrid production can be, for example, a soybean *FAD2-1A* intron suppressed line, a soybean *FAD3-1A, FAD3-1B* and/or *FAD3-1C* intron suppressed line or an intron suppressed line containing *FAD2-1A, FAD3-1A, FAD3-1B* and/or *FAD3-1C* in any desired combination. Any of these parents may be crossed with, for example, any naturally occurring or manmade mutant line with increased levels of oleic acid and/or decreased levels of linolenic acid.

Backcross breeding has been used to transfer genes for a simply inherited, highly heritable trait, such as transgenes, into a desirable homozygous cultivar or inbred line, which is the recurrent parent. The source of the trait to be transferred is called the donor parent. The resulting plant is expected to have the attributes of the recurrent parent (*e.g.*, cultivar) and the desirable trait transferred from the donor parent. After the initial cross, individuals possessing the phenotype of the donor parent are selected and repeatedly crossed (backcrossed) to the

recurrent parent. The resulting parent is expected to have the attributes of the recurrent parent (*e.g.*, cultivar) and the desirable trait transferred from the donor parent. The donor parent in such a backcross production may be, for example, a soybean *FAD2-1A* intron suppressed line or a soybean *FAD3-1A, FAD3-1B* and/or *FAD3-1C* intron suppressed line or an intron suppressed line containing *FAD2-1A, FAD3-1A, FAD3-1B* and/or *FAD3-1C* in any desired combination. The recurrent parent can be for example any naturally occurring or manmade mutant line with increased levels of oleic acid and/or decreased levels of linolenic acid.

Computer Readable Medium

The nucleotide sequence provided in SEQ ID NO: 1 through 15, 18, 19, 22, 23, or fragment thereof or complement thereof, or a nucleotide sequence at least 50%, 60%, or 70% identical, preferably 80%, 85% identical, or especially preferably 90%, or 95% identical, or particularly highly preferably 97%, 98%, or 99% identical to the sequence provided in SEQ ID NO: 1 through 15, 18, 19, 22, 23, or fragment thereof or complement thereof, can be "provided" in a variety of media to facilitate use. Such a medium can also provide a subset thereof in a form that allows a skilled artisan to examine the sequences.

In one application of this embodiment, a nucleotide sequence of the present invention can be recorded on computer readable media. As used herein, "computer readable media" refers to any medium that can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy disk, hard disc, storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. A skilled artisan can readily appreciate how any of the presently known computer readable media can be used to create a manufacture comprising a computer readable medium having recorded thereon a nucleotide sequence of the present invention.

As used herein, "recorded" refers to a process for storing information on computer readable medium. A skilled artisan can readily adopt any of the presently known methods for recording information on computer readable media to generate media comprising the nucleotide sequence information of the present invention. A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a

nucleotide sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable media. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as Word Perfect and Microsoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. A skilled artisan can readily adapt any number of data processor structuring formats (e.g. text file or database) in order to obtain computer readable media having recorded thereon the nucleotide sequence information of the present invention.

By providing one or more nucleotide sequences of the present invention, a skilled artisan can routinely access the sequence information for a variety of purposes. Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium. Software which implements the BLAST (Altschul *et al., J. Mol. Biol. 215*: 403-410 (1990)) and BLAZE (Brutlag *et al., Comp. Chem. 17*:203-207 (1993)) search algorithms on a Sybase system can be used to identify non-coding regions and other nucleic acid molecules of the present invention within the genome that contain homology to non-coding regions from other organisms. Such non-coding regions may be utilized to affect the expression of commercially important proteins such as enzymes used in amino acid biosynthesis, metabolism, transcription, translation, RNA processing, nucleic acid and protein degradation, protein modification, and DNA replication, restriction, modification, recombination, and repair.

The present invention further provides systems, particularly computer-based systems, which contain the sequence information described herein. Such systems are designed to identify commercially important fragments of the nucleic acid molecules of the present invention. As used herein, "a computer-based system" refers to the hardware means, software means, and data storage means used to analyze the nucleotide sequence information of the present invention. The minimum hardware means of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means, and data storage means.

A skilled artisan can readily appreciate that any one of the currently available computer-based systems is suitable for use in the present invention.

As indicated above, the computer-based systems of the present invention comprise a data storage means having stored therein a nucleotide sequence of the present invention and the necessary hardware means and software means for supporting and implementing a search means. As used herein, "data storage means" refers to memory that can store nucleotide sequence information of the present invention, or a memory access means which can access manufactures having recorded thereon the nucleotide sequence information of the present invention. As used herein, "search means" refers to one or more programs which are implemented on the computer-based system to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the sequence of the present invention that match a particular target sequence or target motif. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are available and can be used in the computer-based systems of the present invention. Examples of such software include, but are not limited to, MacPattern (EMBL), BLASTIN, and BLASTIX (NCBIA). One of the available algorithms or implementing software packages for conducting homology searches can be adapted for use in the present computer-based systems.

The most preferred sequence length of a target sequence is from about 10 to 100 amino acids or from about 30 to 300 nucleotide residues. However, it is well recognized that during searches for commercially important fragments of the nucleic acid molecules of the present invention, such as sequence fragments involved in gene expression and protein processing, the target sequence may be of shorter length.

As used herein, "a target structural motif," or "target motif" refers to any rationally selected sequence or combination of sequences in which the sequences are chosen based on a three-dimensional configuration which is formed upon the folding of the target motif. There are a variety of target motifs known in the art. Protein target motifs include, but are not limited to, enzymatic active sites and signal sequences. Nucleic acid target motifs include, but are not

limited to, promoter sequences, *cis* elements, hairpin structures, and inducible expression elements (protein binding sequences).

Thus, the present invention further provides an input means for receiving a target sequence, a data storage means for storing the target sequences of the present invention sequence identified using a search means as described above, and an output means for outputting the identified homologous sequences. A variety of structural formats for the input and output means can be used to input and output information in the computer-based systems of the present invention. A preferred format for an output means ranks fragments of the sequence of the present invention by varying degrees of homology to the target sequence or target motif. Such presentation provides a skilled artisan with a ranking of sequences which contain various amounts of the target sequence or target motif and identifies the degree of homology contained in the identified fragment.

A variety of comparing means can be used to compare a target sequence or target motif with the data storage means to identify sequence fragments sequence of the present invention. For example, implementing software that implement the BLAST and BLAZE algorithms (Altschul *et al., J. Mol. Biol. 215*:403-410 (1990)) can be used to identify non-coding regions within the nucleic acid molecules of the present invention. A skilled artisan can readily recognize that any one of the publicly available homology search programs can be used as the search means for the computer-based systems of the present invention.

The following examples are illustrative and not intended to be limiting in any way.

EXAMPLES

Example 1 Cloning of Desaturase Genomic Sequences

1A. Soybean Δ12 Desaturase (FAD2-1)

A soybean *FAD2-1A* sequence is identified by screening a soybean genomic library using a soybean *FAD2-1* cDNA probe. Three putative soy *FAD2-1* clones are identified and plaque purified. Two of the three soy *FAD2-1* clones are ligated into pBluescript II KS+ (Stratagene) and sequenced. Both genomic clones (14-1 and 11-12) are the same and matched

the corresponding sequence in the soy *FAD2-1* cDNA exactly. A sequence of the entire *FAD2-1A* clone is provided in SEQ ID NO:15.

Prior to obtaining a full length clone, a portion of the *FAD2-1A* genomic clone is PCR amplified using PCR primers designed from the 5' untranslated sequence (Primer 12506, 5'-ATACAA GCCACTAGGCAT-3', SEQ ID NO:16) and within the cDNA (Primer 11698: 5'-GATTGGCCATGCAATGAGGGAAAAGG-3', SEQ ID NO:17). The resulting PCR product is cloned into the vector pCR 2.1 (Invitrogen, Carlsbad,CA.) and sequenced. A soy *FAD2-1A* partial genomic clone (SEQ ID NO:18) with an intron region (SEQ ID NO:1) is identified by comparison to the soybean cDNA sequence using the Pustell comparison program in Macvector. The *FAD2-1A* intron sequence (SEQ ID NO:1) begins after the ATG start codon, and is 420 bases long.

A second *FAD2-1* gene family member is also identified and cloned, and is referred to herein as *FAD2-1B*. The soy *FAD2-1B* partial genomic clone (SEQ ID NO:19) has a coding region (base pairs 1783-1785 and 2191-2463) and an intron region (base pairs 1786-2190) which are identified by comparison to the soybean cDNA sequence using the Pustell comparison program in Macvector. The *FAD2-1B* intron sequence (SEQ ID NO:2) begins after the ATG start codon and is 405 bases long. Other regions in the *FAD2-1B* partial genomic clone (SEQ ID NO: 19) include a promoter (base pairs 1-1704) (SEQ ID NO: 22) and 5'UTR (base pairs 1705-1782).

1B. Soybean Δ15 Desaturase (FAD3)

A partial soybean *FAD3-1A* genomic sequence is PCR amplified from soybean DNA using primers 10632, 5'-CUACUACUACUACTCGAGACAAAGCCTTTAGCCTATG-3' (SEQ ID NO:20), and 10633: 5'-CAUCAUCAUCAUGGATCCCATGTCTCTCTATGCAAG-3' (SEQ ID NO:21). The Expand Long Template PCR system (Roche Applied Sciences, Indianapolis) is used according to the manufacturer's directions. The resulting PCR products are cloned into the vector pCR 2.1 (Invitrogen) and sequenced. A soy *FAD3-1A* partial genomic clone sequence (SEQ ID NO: 23) and intron regions are confirmed by comparisons to the soybean *FAD3-1A* cDNA sequence using the Pustell program in Macvector.

From the identified partial genomic soybean *FAD3-1A* sequence (SEQ ID NO:23), seven introns are identified: *FAD3-1A* intron #1(SEQ ID NO:5), *FAD3-1A* intron #2 (SEQ ID NO:6), *FAD3-1A* intron #3A (SEQ ID NO:7), *FAD3-1A* intron #4 (SEQ ID NO:8), *FAD3-1A* intron #5 (SEQ ID NO:9), *FAD3-1A* intron #3B (SEQ ID NO:10), and *FAD3-1A* intron #3C (SEQ ID NO:11). *FAD3-1A* intron #1 is 191 base pairs long and is located between positions 294 and 484, *FAD3-1A* intron #2 is 346 base pairs long and is located between positions 577 and 922, *FAD3-1A* intron #3A is 142 base pairs long and is located between positions 991 and 1132, *FAD3-1A* intron #3B is 98 base pairs long and is located between positions 1224 and 1321, *FAD3-1A* intron #3C is 115 base pairs long and is located between positions 1509 and 1623, *FAD3-1A* intron #4 is 1228 base pairs long and is located between positions 1707 and 2934, and *FAD3-1A* intron #5 is 625 base pairs long and is located between positions 3075 and 3699.

A partial soybean *FAD3-1B* genomic sequence is PCR amplified from soybean DNA using primers 19386, 5'-GGTAACAGAGAAAGAAACATTTGAGC-3' and 19369: 5'-GCATGCTAACAAAAGTAAGTGC-3'. The Expand Long Template PCR system (Roche Applied Sciences, Indianapolis) is used according to the manufacturer's directions. The resulting PCR products are cloned into the vector pCR 2.1 TOPO (Invitrogen) and sequenced. A soy *FAD3-1B* partial genomic clone sequence and intron regions are confirmed by comparisons to the soybean *FAD3-1B* cDNA sequence using the Pustell program in Sequencher. From the identified partial genomic soybean *FAD3-1B* sequence, seven introns are identified: *FAD3-1B* intron #1, *FAD3-1B* intron #2, *FAD3-1B* intron #3A, *FAD3-1B* intron #4 (SEQ ID NO:13), *FAD3-1B* intron #5, *FAD3-1B* intron #3B, and *FAD3-1B* intron #3C (SEQ ID NO:12).

Example 2 Expression Constructs

2A. Construction of pCGN5468, pCGN5469, pCGN5471, pCGN5485, and pCGN5486

The *FAD2-1A* intron sequence (SEQ ID NO: 1) is amplified via PCR using the *FAD2-1A* partial genomic clone (SEQ ID NO:18) as a template and primers 12701 (5'-ACGAATTCCTCGAGGTAAA TTAAATTGTGCCTGC-3' (SEQ ID NO:24)) and 12702 (5'-GCGAGATCTATCG ATCTGTGTCAAAGTATAAAC-3' (SEQ ID NO:25)). The resulting amplification products are cloned into the vector pCR 2.1 (Invitrogen) and sequenced. The

FAD2-1A intron is then cloned into the expression cassette, pCGN3892, in sense and antisense orientations. The vector pCGN3892 contains the soybean 7S promoter and a pea RBCS 3'. Both gene fusions are then separately ligated into pCGN9372, a vector that contains the CP4 gene regulated by the FMV promoter. The resulting expression constructs (pCGN5469 sense (Figure 2) and pCGN5471 antisense (Figure 3)) are used for transformation of soybean using biolistic methods described below.

The *FAD2-1B* intron sequence (SEQ ID NO: 2) is amplified via PCR using the *FAD2-1B* partial genomic clone (SEQ ID NO: 19) as a template and primers 13883 (5'-GCGATCGATGTATGATGCTAAATTAAATTGTGCCTG-3' (SEQ ID NO:28)) and 13876 (5'-GCGGAATTCCTGTGTCAAAGTATAAAGAAG-3' (SEQ ID NO:29)). The resulting amplification products are cloned into the vector pCR 2.1 (Invitrogen) and sequenced. The *FAD2-1B* intron is fused to the 3' end of the *FAD2-1A* intron in plasmids pCGN5468 (Figure 1) (containing the soybean 7S promoter fused to the *FAD2-1A* intron (sense) and a pea RBCS 3') or pCGN5470 (containing the soybean 7S promoter fused to the *FAD2-1A* intron (antisense) and a pea RBCS 3') in sense or antisense orientation, respectively. The resulting intron combination fusions are then ligated separately into pCGN9372, a vector that contains the CP4 gene regulated by the FMV promoter. The resulting expression constructs (pCGN5485 (Figure 4), *FAD2-1A* & *FAD2-1B* intron sense; and pCGN5486 (Figure 5), *FAD2-1A* & *FAD2-1B* intron antisense) are used for transformation of soybean using biolistic methods described below.

2B. PCR amplification of FAD3-1A introns

Four of the seven introns identified from the soybean *FAD3-1A* genomic clone are PCR amplified using the *FAD3-1A* partial genomic clone as template and primers as follows: *FAD3-1A* intron #1, primers 12568: 5'-GATCGATGCCCGGGGTAATAATTTTTGTGT-3' (SEQ ID NO:30) and 12569: 5'-CACGCCTCGAGTGTTCAATTCAATCAATG-3' (SEQ ID NO:31); *FAD3-1A* intron #2, primers 12514: 5'-CACTCGAGTTAGTTCATACTGGCT-3' (SEQ ID NO:32) and 12515: 5'-CGCATCGATTGCAAAATCCATCAAA-3' (SEQ ID NO:33); *FAD3-1A* intron #4, primers 10926: 5'-CUACUACUACUACTCGAGCGTAAATAGTGGGTGAACAC-3' (SEQ ID NO:34) and

10927: 5'-CAUCAUCAUCAUCTCGAGGAATTCGTCCATTTTAGTACACC-3' (SEQ ID NO:35); *FAD3-1A* intron #5, primers 10928: 5'-CUACUACUACUACTCGAGGCGCGT ACATTTTATTGCTTA-3' (SEQ ID NO:36) and 10929: 5'-CAUCAUCAUCAUCT CGAGGAATTCTGCAGTGAATCCAAATG-3' (SEQ ID NO:37). The resulting PCR products for each intron are cloned into the vector pCR 2.1 (Invitrogen) and sequenced.

2C. Construction of pCGN5455, pCGN5459, pCGN5456, pCGN5460, pCGN5466, and pCGN5473

FAD3-1A introns #1, #2, #4 and #5 are each ligated separately into pCGN3892, in sense or antisense orientations. pCGN3892 contains the soybean 7S promoter and a pea RBCS 3'. These fusions are ligated into pCGN9372, a vector that contains the CP4 gene regulated by the FMV promoter for transformation into soybean. The resulting expression constructs (pCGN5455 (Figure 12), *FAD3-1A* intron #4 sense; pCGN5459 (Figure 13), containing *FAD3-1A* intron #4 antisense; pCGN5456, *FAD3* intron #5 sense; pCGN5460, *FAD3-1A* intron #5 antisense; pCGN5466 (Figure 7), containing *FAD3-1A* intron #2 antisense; pCGN5473 (Figure 9), containing *FAD3-1A* intron #1 antisense) are used for transformation of soybean using biolistic methods described below.

Introns #3C and #4 are also PCR amplified from a second *FAD3* gene family member (*FAD3-1B*). Soybean *FAD3-1B* introns #3C and #4 are PCR amplified from soybean DNA using the following primers, 5' CATGCTTTCTGTGCTTCTC 3' (SEQ ID NO:26) and 5' GTTGATCCAACCATAGTCG 3' (SEQ ID NO:27). The PCR products are cloned into the vector pCR 2.1 (Invitrogen) and sequenced. Sequences for *FAD3-1B* introns #3C and #4 are provided in SEQ ID NOs:12 and 13, respectively.

Intron #4 from three soybean *FAD3* gene family members, *FAD3-1A, FAD3-1B* and *FAD3-1C* is PCR amplified. Intron #4 from the *FAD3-1A* gene is PCR amplified using the *FAD3-1A* partial genomic clone as template and primers primers 10926: 5'-CUACUACUACUACTCGAGCGTAAATAGTGGGTGAACAC-3' (SEQ ID NO:34) and 10927: 5'-CAUCAUCAUCAUCTCGAGGAATTCGTCCATTTTAGTACACC-3' (SEQ ID NO:35). Intron #4 from the *FAD3-1B* gene is PCR amplified using the soybean genomic DNA as template and primers #17823: GTATCCCATTTAACAC and #17824:

CTGTGAAATTACATATAG. Intron #4 from the *FAD3-1C* gene is PCR amplified using the soybean genomic DNA as template and primers #17826: GCGCCGCTCGAGCTGTCCATTTTTGTACAC and #17825: CCGGCGCTCGAGGTAACAAAAATAAATAGAAAATAGTGAGTG. The resulting PCR products for each intron were cloned into the vector pCR 2.1 (Invitrogen) and sequenced. *FAD3-1A* intron, #4 was ligated into the pCGN3892 in sense orientation. The resulting expression cassette, pCGN5453, contained the 7S alpha' promoter fused to the *FAD3-1A* intron #4, with a pea RBCS 3'.

2D. Construction of pMON68521

To construct pMON68521 (Figure 10), pCGN5453 (7S alpha' promoter fused to the *FAD3-1 A* intron #4, with a pea RBCS 3') and KWHIT 032858 (PCR2.1 containing the *FAD3-1B* intron #4) are digested with EcoRI and ligated together to form KWHIT03004 (7Salpha' promoter fused to the *FAD3-1A* intron #4 and *FAD3-1B* intron #4, with a pea RBCS 3'). KWHIT03004 is digested with XhoI and KAWHIT032980 (*FAD*3-1C intron #4 in PCR2.1) is digested with EcoRI and the ends of both digested plasmids are filled and subsequently ligated. The resulting plasmid is KAWHIT030005 (7Salpha' promoter fused to the *FAD*3-1A intron #4, *FAD3-1B* intron #4, and *FAD3*-1B intron #4, with a pea RBCS 3'). KAWHIT030005 is digested with SacI and the ends are filled using the Klenow fragment of T4 polymerase to create blunt ends. pCGN5468 (Figure 1, containing 7S alpha' promoter fused to the *FAD*2-1A intron, with a pea RBCS 3') is digested with EcoRI and the ends are filled with Klenow fragment of T4 polymerase to form blunt ends. The blunt ends of plasmids KAWHIT030005 and pCGN5468 are ligated together to form KWHIT03001 (7S alpha' promoter fused to the *FAD*2-1A intron, the *FAD*3-1A intron #4, *FAD*3-1B intron #4, *FAD*3-1C intron #4, with a pea RBCS 3'). KWHIT03001 and pMON 70276 (FMV-EF-1/CP4) are both digested with NotI, ligated together to form pMON68521 (Figure 10, 7S alpha' promoter fused to the *FAD*2-1A intron, the *FAD*3-1A intron #4, *FAD*3-1B intron #4, *FAD*3-1C intron #4, with a pea RBCS 3' and FMV-EF-1/CP4, with a pea RBCS 3'). pMON68521 was transformed into the ABI strain of *Agrobacterium tumefaciens* and cocultivated into soybean.

2E. Construction of pMON68519

In constructing the plasmid pMON68519 (Figure 11), pCGN5453 (containing 7Salpha' promoter fused to the *FAD3-1A* intron #4, with a pea RBCS 3') and KWHIT 032858 (pCR2.1 containing the *FAD1B* intron #4) are digested with EcoRI and ligated together to form KWHIT03004 (containing 7Salpha' promoter fused to the *FAD3-1A* intron #4 and *FAD3-1B* intron #4, with a pea RBCS 3'). KWHIT03004 is digested with XhoI and KAWHIT032980 (containing *FAD*3-1C intron #4 in PCR2.1) is digested with EcoRI. The ends of both digested plasmids are filled and the plasmids are subsequently ligated. The resulting plasmid is named KAWHIT030005 (containing 7Salpha' promoter fused to the *FAD*3-1A intron #4, *FAD3-1B* intron #4, and *FAD3*-1B intron #4, with a pea RBCS 3'). KAWHIT030005 is digested with SacI and then the DNA is treated with the Klenow fragment of T4 polymerase to create blunt ends. pCGN7770 (containing the *Napin* promoter and *Napin* 3') is digested with XhoI and blunt-ended with Klenow fragment of T4 polymerase. The blunt ends of plasmids KAWHIT030005 and pCGN7770 are ligated together to form KWHIT03007 (containing a *Napin* promoter fused to the *FAD*2-1A intron, the *FAD*3-1A intron #4, *FAD*3-*1B* intron #4, *FAD*3-1C intron #4, with a *Napin* 3').

KWHIT03007 is then digested with NotI and the ends are filled with the Klenow fragment of T4 polymerase, forming blunt ends. The *Napin* promoter fused to the *FAD*2-1A intron, the *FAD*3-1A intron #4, *FAD*3-1B intron #4, *FAD*3-1C intron #4, with a *Napin* 3' (KWHIT03007) is blunt end ligated into pMON68504 which is digested with EcoRV. pMON68504 contains the Soy *FAD2-1A* intron fused to the 7s Alpha' promoter within the 2 tDNA vector pMON41162, which contains the CP4 gene regulated by the FMV promoter, with the pea RBCS 3'. The blunt end ligation between KWHIT03007 and pMON6850 results in the plasmid, pMON68519. pMON68519 is transformed into the ABI strain of *Agrobacterium tumefaciens* and cocultivated into soybean.

Example 3 Plant Transformation and Analysis

Linear DNA fragments containing the expression constructs for sense and antisense suppression of the Δ12 and Δ15 desaturase genes are stably introduced into soybean (Asgrow

variety A3244 or A4922A32) by the particle bombardment method of McCabe *et al.* (1988), *Bio/Technology, 6*:923-926 or via cocultivation with *Agrobacterium tumefaciens*, strain ABI. (Martinell, U.S. Patent No. 6,384,310). Transformed soybean plants are identified by selection on media containing glyphosate.

Fatty acid compositions are analyzed from seed of soybean lines transformed with the intron expression constructs using gas chromatography. R1 pooled seed and R1 single seed oil compositions demonstrate that the mono- and polyunsaturated fatty acid compositions were altered in the oil of seeds from transgenic soybean lines as compared to that of the seed from non-transformed soybean. Tables I, II, and III provide summaries of results which were obtained using the described constructs. These data show that sense and antisense expression of non-coding regions of a desaturase gene result in the modification of the fatty acid compositions. The data also shows that introns can be used to obtain a variety of lines with varying fatty acid compositions. Selections can be made from such lines depending on the desired relative fatty acid composition. Because each intron is able to modify the levels of each fatty acid to varying extents, it is contemplated that combinations of introns can be used depending on the desired compositions.

TABLE I

			Oleic	**Linoleic**	**Linolenic**
FAD2	**Orientation**	**Event**			
wildtype (control)		5469-5 null R1 pool	18.15%	55.59%	7.97%
		10 seed average	13.89%	55.89%	9.067%
		5469-27 null R1 pool	19.15%	54.62%	9.32%
		A4922	15.75%	56.1%	8.75%
		5471-13 null R1 pool	17.02%	56.49%	9.08%
		10 seed average	13.86%	56.14%	9.49%
		A4922	14.95%	55.95%	9.07%
full length cDNA (control)	sense	5462-133 R1 pool	84%	2.17%	1.55%
		best 5462-133 R1 seed	84%	0.59%	1.76%
intron 1	sense	5469-6 R1 pool	29.93%	46.53%	
		5469-8 R1 pool	36.5%	42.11%	5.98%
		best 5469-6 R1 seed	44.41%	29.34%	6.68%
		best 5469-8 R1 seed	41.26%	33.16%	5.74%
		5469-14 R1 pool	61.06%	16.42%	7.75%
		5469-20 R1 pool	48.89%	31.61%	4.89%
		5469-22 R1 pool	80%	2.97%	4.78%
		best 5469-14 R1 seed	62.21%	11.97%	8.81%
		5485-3 R1 pool	63.54%	14.09%	7.32%
		5485-53 R1 pool	47.58%	27.64%	7.81%

			Oleic	Linoleic	Linolenic
FAD2	**Orientation**	**Event**			
	antisense	5471-8 R1 pool	31.05%	43.62%	7.07%
		5471-2 R1 pool	27.98%	48.88%	6.83%
		5471-26 R1 pool	32.66%	44.54%	6.76%
		best 5471-8 R1 seed	57.4%	23.37%	5.73%
		best 5471-2 R1 seed	28.08%	46.14%	6.52%
		best 5471-26 R1 seed	43.3%	34.15%	5.6%
		5486-33 R1 pool	32.37%	43.66%	6.87%
		5486-12 R1 pool	27.32%	46.97%	6.4%
		5486-40 R1 pool	26.79%	48.72%	6.55%
FAD3					
wildtype (control)		**5473-7 null R1 pool**	**15.65%**	**56.74%**	**9.55%**
		A4922 R1 pool	**19.84%**	**56.79%**	**7.48%**
full length cDNA (control)	**sense**	**5464-50 R1 pool**	**18.06%**	**62.03%**	**2.75%**
		best 5464-50 R1 seed	**17.08%**	**62.44%**	**1.72%**
intron 1	antisense	5473-8 R1 pool	33.47%	45.97%	5.54%
		5473-1 R1 pool	33.34%	42.67%	7.59%
intron 2	antisense	5466-20 R1 pool	28.43%	48.83%	6.37%
		5466-16 R1 pool	27.61%	49.92%	5.96%
intron 4	sense	5455-19 R1 pool	40.35%	39.97%	4.61%
		5455-10 R1 pool	35.14%	43.59%	5.53%
		5455-57 R1 pool	38.04%	42.44%	5.24%
		5455-76 R1 pool	37.24%	42.42%	5.37%
		5455-107 R1 pool	36.44%	42.72%	5.62%
		best 5455-57 R1 seed	45.36%	35.55%	4.92%
		best 5455-76 R1 seed	35.3%	43.54%	5.53%
		best 5455-107 R1 seed	45.56%	34.85%	5.12%
	antisense	5459-2 R1 pool	34.5%	43.87%	5.59%
		5459-6 R1 pool	33.78%	44.12%	5.62%
		5459-20 R1 pool	28.26%	49.48%	5.5%
		best 5459-2 R1 seed	61.45%	23.45%	3.38%
		best 5459-6 R1 seed	53.51%	29.68%	3.53%
		best 5459-20 R1 seed	30%	50.55%	4.15%
intron 5	sense	5456-38 R1 pool	28.23%	49.59%	6.74%
		5456-62 R1 pool	28.94%	48.66%	6.25%
		best 5456-62 R1 seed	29.5%	43.69%	5.4%
	antisense	5460-9 R1 pool	29.78%	48.57%	5.54%
		5460-21 R1 pool	28.37%	49.79%	5.54%
		best 5460-21 R1 seed	35.18%	40.52%	5.33%

TABLE II Oil Composition data for seeds containing pMON68521

R1 single seed data

Construct	Strain ID	16:0	18:0	18:1	18:2	18:3
PMON68521	GM_A32162	12.0	3.4	42.8	35.5	5.3
PMON68521	GM_A32162	11.5	2.6	39.4	40.0	5.2
PMON68521	GM_A31619	10.4	2.8	39.1	40.4	5.8
PMON68521	GM_A32162	11.9	2.6	36.7	41.9	5.8
PMON68521	GM_A32162	12.2	2.5	34.9	43.0	6.3
PMON68521	GM_A32162	13.0	2.8	30.4	46.6	6.0
PMON68521	GM_A31610	12.4	1.9	28.3	49.8	7.1
PMON68521	GM_A32162	11.9	2.9	26.5	51.6	6.1
PMON68521	GM_A31792	13.2	3.3	25.3	50.2	7.2
PMON68521	GM_A31395	12.5	3.7	25.1	50.2	6.7
PMON68521	GM_A31393	13.1	3.5	24.1	51.9	5.6
PMON68521	GM_A31615	14.0	2.2	24.0	52.5	6.7
PMON68521	GM_A32209	12.5	3.5	23.7	51.5	7.6
PMON68521	GM_A31612	11.8	3.0	23.7	51.1	9.5

Construct	Strain ID	16:0	18:0	18:1	18:2	18:3
PMON68521	GM_A32209	12.6	3.3	23.6	52.2	7.2
PMON68521	GM_A32209	12.4	3.2	23.0	53.1	7.2
PMON68521	GM_A31489	12.3	3.2	22.5	54.0	6.9
PMON68521	GM_A32252	12.7	4.4	22.3	52.4	7.1
PMON68521	GM_A32162	12.6	3.1	22.2	54.9	6.2
PMON68521	GM_A32089	13.5	3.2	22.2	52.4	7.9
PMON68521	GM_A31393	12.6	4.3	22.2	53.0	5.7
PMON68521	GM_A31610	12.5	2.7	21.8	55.5	6.9
PMON68521	GM_A31610	12.6	2.4	21.7	55.8	6.9
PMON68521	GM_A31656	12.7	3.6	21.6	53.2	8.0
PMON68521	GM_A31612	12.3	3.6	21.6	54.0	7.2
PMON68521	GM_A31610	13.2	2.6	21.0	56.2	6.5
PMON68521	GM_A31604	13.5	3.1	20.4	55.3	7.0
PMON68521	GM_A31610	13.2	2.6	20.4	56.7	6.4
PMON68521	GM_A31489	12.8	3.1	20.1	55.5	7.1
PMON68521	GM_A31525	12.2	3.0	20.1	57.2	6.3
A3244		13.9	4.1	15.8	56.3	9.0
A3244		13.7	4.1	14.2	57.6	9.3
A3244		13.6	4.3	14.1	57.4	9.7
A3244		13.9	4.1	14.1	56.9	10.0
A3244		13.8	4.4	13.6	57.6	9.8
A3244		14.2	4.8	13.6	56.8	9.5
A3244		14.2	4.3	13.2	56.5	10.8
A3244		14.0	4.2	13.1	57.0	10.6
A3244		14.0	4.5	12.9	57.3	10.3

TABLE III Oil Composition data for seeds containing pMON68519

R1 single seed data

Construct	Strain ID	16:0	18:0	18:1	18:2	18:3
PMON68519	GM_A29911	13.0	4.8	40.7	34.4	5.2
PMON68519	GM_A29911	12.3	3.8	38.8	37.5	5.8
PMON68519	GM_A29911	12.3	3.3	34.0	42.6	6.5
PMON68519	GM_A32856	12.9	3.5	33.6	42.2	6.5
PMON68519	GM_A32856	12.6	3.1	33.3	43.4	6.4
PMON68519	GM_A32856	13.0	3.1	31.3	45.4	6.1
PMON68519	GM_A32856	12.7	3.2	28.9	47.3	6.6
PMON68519	GM_A29911	12.9	4.0	28.7	46.5	6.7
PMON68519	GM_A29911	12.2	3.1	28.1	47.4	6.8
PMON68519	GM_A32856	13.3	3.2	26.5	48.7	7.1
PMON68519	GM_A32856	13.2	3.2	26.5	49.3	6.8
PMON68519	GM_A29911	13.1	3.2	26.1	49.4	7.1
PMON68519	GM_A32856	13.2	3.6	25.9	48.9	7.1
PMON68519	GM_A32857	13.3	3.1	23.3	52.2	7.4
PMON68519	GM_A29911	12.8	3.5	23.3	52.5	6.6
PMON68519	GM_A29911	12.6	3.1	21.4	51.9	9.0
PMON68519	GM_A29911	13.4	3.6	20.1	53.6	7.9

Construct	Strain ID	16:0	18:0	18:1	18:2	18:3
A3244		13.2	4.3	16.5	55.7	9.7
A3244		13.5	3.2	16.3	56.8	9.2
A3244		13.7	3.4	15.4	57.7	9.2
A3244		13.9	3.3	15.1	57.9	9.1
A3244		13.8	3.6	14.3	58.3	9.2
A3244		13.5	3.4	13.6	58.2	10.4
A3244		13.5	3.9	12.5	58.4	11.2
A3244		14.6	3.8	12.1	58.2	10.8
A3244		14.5	3.9	11.8	57.8	11.4

Example 4

Linear DNA fragments containing the dsRNAi constructs for suppression of the Δ12 and Δ15 desaturase genes are stably introduced into soybean (Asgrow variety A3244 or A4922A32) via cocultivation with *Agrobacterium tumefaciens*, strain ABI. (Martinell, U.S. Patent No. 6,384,301) or by the particle bombardment method of McCabe *et al.* (1988), *Bio/Technology, 6*:923-926. Introduced constructs include: (1) 7S promoter - *FAD2-1A* sense intron - *FAD3-1A* sense intron - *FAD3-1B* sense intron - spliceable *FAD3* intron #5 - *FAD3-1B* antisense intron - *FAD3-1A* antisense intron - *FAD2-1A* antisense intron- pea rbcS; (2) 7S promoter - *FAD3-1A* sense intron - *FAD3-1B* sense intron- spliceable *FAD3* intron #5 - *FAD3-1B* antisense intron - *FAD3-1A* antisense intron - pea rbcS; (3) 7S promoter - *FAD2-1A* sense intron - *FAD3-1A* sense intron - spliceable *FAD3* intron #5 - *FAD3-1A* antisense intron - *FAD2-1A* antisense intron - pea rbcS. Representative sequences for *FAD2-1A*, *FAD2-1B*, *FAD2-2B*, *FAD3-1A*, *FAD3-1B*, and *FAD3-1C* introns may be found, without limitation, in U.S. Application Serial Number 10/176,149, filed June 21, 2002, and U.S. Patent Application Serial Number 09/638,508, filed August 11, 2000, and U.S. Provisional Application Serial Number 60/151,224, filed August 26, 1999, and U.S. Provisional Application Serial Number 60/172,128, filed December 17, 1999. Transformed soybean plants are identified by selection on media containing glyphosate.

Fatty acid compositions are analyzed from transformed soybean seeds containing inron RNAi suppression constructs. Particular lines are selected depending on the desired relative fatty acid composition.

Example 5

5A.

RNA is isolated from homozygous R2 seed from two *FAD2-1* intron suppressed lines (5469-14 and 5469-22), from two *FAD2-1* cDNA suppressed lines (positive controls) (5462-87 and 5462-133), and from negative controls (wild type seed and seed from null segregants from each intron suppressed event). Northern gels containing these RNA samples are probed with the *FAD2-1* cDNA. *FAD2-1A* transcript levels are significantly reduced in both the intron suppressed lines and the cDNA suppressed lines relative to the negative controls. The same Northern blot is probed with the constitutive *FAD2-2* cDNA and no significant differences in the *FAD2-2* transcript levels are observed between the *FAD2-1* intron suppressed lines and the controls. In contrast, the *FAD2-2* transcript in the cDNA suppressed lines, is significantly reduced. This Northern data indicates that the *FAD2-1A* intron is specifically inhibiting the accumulation of the *FAD2-1* transcript but not the *FAD2-2* transcript. A partial *FAD2-2* genomic clone (SEQ ID NO: 3) is PCR amplified and sequence analysis reveals a 4.7 KB intron in the 5' untranslated region of the gene. The sequence of the *FAD2-2* intron (SEQ ID NO: 4) shares no homology with the *FAD2-1* intron.

5B.

RNA is isolated from homozygous R2 seed from four *FAD3-1A* intron #4 suppressed lines, from three *FAD3-1B* intron #4 suppressed lines, from negative control seed (non transformed wild type seed) and from seed from null segregants from each intron suppressed event). Northern gels containing these RNA samples are probed with the *FAD3-1A* 3'UTR region. Endogenous *FAD3-1A* transcript levels are significantly reduced in the *FAD3-1A* intron #4 suppressed lines relative to the wildtype or null controls. The same Northern blot is probed with the *FAD3-1B* 3' UTR region and no significant differences in the endogenous *FAD3-1B* transcript levels are observed relative to the *FAD3-1A* intron #4 suppressed lines, the wildtype or null controls. The sequence of the *FAD3-1A* intron #4 (SEQ ID NO: 8) shares no homology with the *FAD3-1B* intron #4 (SEQ ID NO: 13)

Example 6

Southern blot data indicate that there are at least two *FAD3* gene family members. To determine the sequence of the other *FAD3* gene family member and to determine if other members exist, a *FAD3-1A* gene sequence is used for a query Blast search against the Monsanto soybean DNA sequences database. Candidate ESTs from different *FAD3* gene family members are used to design primers. Using this strategy, 2 primer sets are designed based on putative *FAD3* sequences. Intron #4 regions from two other *FAD3* gene family members are isolated. Primers are designed from the 211565_1.r1040 EST (designated *FAD3-1B*), (5' primer #15024: 5'-CATGCTTTCTGTGCTTCTC-3' (SEQ ID NO:26) and 3' primer #15027: 5'-GTTGATCCAACCATAGTCG-3' (SEQ ID NO:27)) in the region corresponding to the position of intron #4 of the *FAD3-1A* gene. These primers are used to PCR amplify the *FAD3-1B* intron #4 (SEQ ID NO:13), which when sequenced shared no sequence homology with the *FAD3-1A* intron #4 (SEQ ID NO: 8). The *FAD3-1B* gene also contains an intron #3C (SEQ ID NO: 12), which also did not share any homology with the *FAD3-1A* intron #3C (SEQ ID NO:11).

Another additional intron #4 is PCR amplified from a second EST, gsv701051989.H1 (designated *FAD3-1C*) using the following set of primers: 5' primer #16241: 5'-CACCATGGTCATCATCAGAAAC (SEQ ID NO:38) and the 3' primer #16242: TCACGATCCACAGTTGTGAGAC (SEQ ID NO:39). The *FAD3-1C* intron #4 (SEQ ID NO:14) shares 50% homology with the *FAD3-1A* intron #4 (SEQ ID NO: 8) and shares no homology with the *FAD3-1B* intron #4 (SEQ ID NO: 13). The *FAD3-1C* EST, like the *FAD3-1B* EST, also contains an intron #4 splice site in the same region of the gene.

Example 7 *FAD2-1A/FAD3-1A* Transformed Plants

7A.

A soybean *FAD2-1A* intron suppressed line is used to pollinate a soybean *FAD3-1A* intron suppressed line that is generated according to the methodology set forth in Example 3. RNA from soybean seeds containing both an expressed *FAD2-1A* intron region and *FAD3-1A* intron region is screened using Northern blotting (as described in Example 5) to determine the levels of *FAD2-1*, *FAD2-2*, *FAD3-1A* and *FAD3-1B* transcripts. Soybean plants with

undetectable or low levels of *FAD2-1* and *FAD3-1A* transcripts are screened for fatty acid composition as set forth in Example 3.

7B.

A soybean *FAD2-1A* intron suppressed line is also used to pollinate a low linolenic soybean *FAD3* mutant line derived from a mutation. RNA from soybean seeds containing both one ore more expressed *FAD2* intron regions and *FAD3* mutations, including knock out, are screened using Northern blots (as described in Example 5) to determine the levels of *FAD2-1*, *FAD2-2*, *FAD3-1A*, *FAD3-1C* and *FAD3-1B* transcripts. Soybean plants with undetectable or low levels of *FAD2* and *FAD3* transcripts are screened for fatty acid composition as set forth in Example 3.

7C.

A soybean line with *FAD3-1A, FAD3-1B, and FAD3-1C* intron suppression is used to pollinate soybean plants with elevated levels of oleic acid containing a *FAD2* mutant line derived from a spontaneous mutation. Soybean lines with *FAD3-1A* and *FAD3-1B* intron suppression, lines with *FAD3-1A* and *FAD3-1C* intron suppression, lines with *FAD3-1B* and *FAD3-1C* intron suppression, lines with *FAD3-1A* intron suppression, lines with *FAD3-1B* intron suppression and lines with *FAD3-1C* intron suppression are each used individually to pollinate soybean plants with elevated levels of oleic acid containing a *FAD2* mutant line derived from a spontaneous mutation. RNA from soybean seeds containing both one or more expressed *FAD3* intron regions and *FAD2* mutations, including knock out, are screened using Northern blots as described in Example 5 to determine the levels of *FAD2-1*, *FAD2-2*, *FAD3-1A,*, *FAD3-1B* and *FAD3-1C* transcripts. Soybean plants with undetectable or low levels of *FAD*2 or *FAD3* transcripts are screened for fatty acid composition as set forth in Example 3.

Example 8 Single *FAD2/FAD3* Constructs

Linear DNA fragments containing sense and antisense *FAD2* and *FAD3* introns, as well as *FAD2* and *FAD3* introns capable of producing a dsRNA, are constructed as set forth in Table IV.

TABLE IV

Construct No.	Promoter 1	Structural Nucleic Acid 1 (sense, antisense, dsRNA)	Promoter 2	Structural Nucleic Acid 2 (sense, antisense, dsRNA)
1	CaMV	*FAD2-1A* intron 1	CaMV	*FAD3-1A* intron 1
2	CaMV	*FAD2-1B* intron 1	CaMV	*FAD3-1A* intron 1
3	CaMV	*FAD2-1A* intron 1	CaMV	*FAD3-1A* intron 4
4	CaMV	*FAD2-1B* intron 1	CaMV	*FAD3-1A* intron 4
5	CaMV	*FAD2-1A* intron 1	CaMV	*FAD3-1B* intron 4
6	CaMV	*FAD2-1B* intron 1	CaMV	*FAD3-1B* intron 4
7	CaMV	*FAD2-1A* intron 1	CaMV	*FAD3-1C* intron 4
8	CaMV	*FAD2-1B* intron 1	CaMV	*FAD3-1C* intron 4
9	CaMV	*FAD2-1A* intron 1	CaMV	*FAD2-2B* intron 1
10	CaMV	*FAD2-1B* intron 1	CaMV	*FAD2-2B* intron 1
11	napin	*FAD2-1A* intron 1	napin	*FAD3-1A* intron 1
12	napin	*FAD2-1B* intron 1	napin	*FAD3-1A* intron 1
13	napin	*FAD2-1A* intron 1	napin	*FAD3-1A* intron 4
14	napin	*FAD2-1B* intron 1	napin	*FAD3-1A* intron 4
15	napin	*FAD2-1A* intron 1	napin	*FAD3-1B* intron 4
16	napin	*FAD2-1B* intron 1	napin	*FAD3-1B* intron 4
17	napin	*FAD2-1A* intron 1	napin	*FAD3-1C* intron 4
18	napin	*FAD2-1B* intron 1	napin	*FAD3-1C* intron 4
19	napin	*FAD2-1A* intron 1	napin	*FAD2-2B* intron 1
20	napin	*FAD2-1B* intron 1	napin	*FAD2-2B* intron 1
21	7S	*FAD2-1A* intron 1	CaMV	*FAD3-1A* intron 1
22	7S	*FAD2-1B* intron 1	CaMV	*FAD3-1A* intron 1
23	7S	*FAD2-1A* intron 1	CaMV	*FAD3-1A* intron 4
24	7S	*FAD2-1B* intron 1	CaMV	*FAD3-1A* intron 4
25	7S	*FAD2-1A* intron 1	CaMV	*FAD3-1B* intron 4
26	7S	*FAD2-1B* intron 1	CaMV	*FAD3-1B* intron 4
27	7S	*FAD2-1A* intron 1	CaMV	*FAD3-1C* intron 4
28	7S	*FAD2-1B* intron 1	CaMV	*FAD3-1C* intron 4
29	7S	*FAD2-1A* intron 1	CaMV	*FAD2-2B* intron 1
30	7S	*FAD2-1B* intron 1	CaMV	*FAD2-2B* intron 1
31	CaMV	*FAD2-1A* intron 1	7S	*FAD3-1A* intron 1
32	CaMV	*FAD2-1B* intron 1	7S	*FAD3-1A* intron 1
33	CaMV	*FAD2-1A* intron 1	7S	*FAD3-1A* intron 4
34	CaMV	*FAD2-1B* intron 1	7S	*FAD3-1A* intron 4
35	CaMV	*FAD2-1A* intron 1	7S	*FAD3-1B* intron 4
36	CaMV	*FAD2-1B* intron 1	7S	*FAD3-1B* intron 4
37	CaMV	*FAD2-1A* intron 1	7S	*FAD3-1C* intron 4
38	CaMV	*FAD2-1B* intron 1	7S	*FAD3-1C* intron 4
39	CaMV	*FAD2-1A* intron 1	7S	*FAD2-2B* intron 1
40	CaMV	*FAD2-1B* intron 1	7S	*FAD2-2B* intron 1

As shown, each construct listed in the table can have several configurations depending on the nature and orientation of the structural nucleic acids in the construct. For example, construct 30 may be configured as follows: (1) 7S promoter - *FAD2-1B* intron 1 (sense) - CaMV promoter - *FAD2-2B* intron 1 (sense); (2) 7S promoter - *FAD2-1B* intron 1 (sense) - CaMV promoter - *FAD2-2B* intron 1 (antisense); (3) 7S promoter - *FAD2-1B* intron 1 (sense) - CaMV promoter - *FAD2-2B* intron 1 (dsRNA); (4) 7S promoter - *FAD2-1B* intron 1 (antisense) - CaMV promoter - *FAD2-2B* intron 1 (sense); (5) 7S promoter - *FAD2-1B* intron 1 (antisense) - CaMV promoter - *FAD2-2B* intron 1 (antisense); (6) 7S promoter - *FAD2-1B* intron 1 (antisense) - CaMV promoter - *FAD2-2B* intron 1 (dsRNA); (7) 7S promoter - *FAD2-1B* intron 1 (dsRNA) - CaMV promoter - *FAD2-2B* intron 1 (sense); (8) 7S promoter - *FAD2-1B* intron 1 (dsRNA) - CaMV promoter - *FAD2-2B* intron 1 (antisense); or (9) 7S promoter - *FAD2-1B* intron 1 (dsRNA) - CaMV promoter - *FAD2-2B* intron 1 (dsRNA).

These constructs can be stably introduced into soybean (for example, Asgrow variety A4922 or Asgrow variety A3244) by the methods described earlier, including the particle bombardment method of McCabe *et al.* (1988), *Bio/Technology, 6*:923-926 or *Agrobacterium*-mediated transformation (Martinell, U.S. Patent No. 6,384,301). Transformed soybean plants are identified by selection on media containing glyphosate. Fatty acid compositions are analyzed from seed of soybean lines transformed with the constructs using gas chromatography.

Example 9

Linear DNA fragments containing expression constructs for sense and antisense expression of the *FAD2-1* and *FAD2-2* introns are stably introduced into soybean (for example, Asgrow variety A4922 or Asgrow variety A3244) by the methods described earlier, including the particle bombardment method of McCabe *et al.* (1988), *Bio/Technology, 6*:923-926 or *Agrobacterium*-mediated transformation (Martinell, U.S. Patent No. 6,384,301). The following constructs are introduced: (1) *FAD2-1A* intron (sense) - *FAD2-2* intron (antisense); (2) *FAD2-1A* intron (sense) - *FAD2-2* intron (sense); (3) *FAD2-1A* intron (antisense) - *FAD2-2* intron (antisense); (4) *FAD2-1A* intron (antisense) - FAD*2-2* intron (sense); (5) *FAD2-1B* intron (sense) - *FAD2-2* intron (antisense); (6) *FAD2-1B* intron (sense) - *FAD2-2* intron (sense); (7)

FAD2-1B intron (antisense) - *FAD2-2* intron (antisense); and (8) *FAD2-1B* intron (antisense) - *FAD2-2* intron (sense). Transformed soybean plants are identified by selection on media containing glyphosate. Fatty acid compositions are analyzed from seed of soybean lines transformed with the constructs using gas chromatography. Seed of the transformed plants exhibit high levels of oleic acid (over 80%).

Additional linear DNA fragments containing expression constructs for sense and antisense expression of the *FAD2-1, FAD2-2*, and *FAD3* introns are stably introduced into soybean (Asgrow variety A4922) by the method of McCabe *et al.* (1988), *Bio/Technology, 6*:923-926. Exemplary constructs include: (1) *FAD2-1A* intron (sense or antisense) - *FAD2-2* intron (sense or antisense) - *FAD3-1A* intron 1 (sense or antisense); (2) *FAD2-1A* intron (sense or antisense) - *FAD2-2* intron (sense or antisense) - *FAD3-1A* intron 4 (sense or antisense); (3) *FAD2-1A* intron (sense or antisense) - *FAD2-2* intron (sense or antisense) - *FAD3-1B* intron 4 (sense or antisense); and (4) *FAD2-1A* intron (sense or antisense) - *FAD2-2* intron (sense or antisense) - *FAD3-1C* intron 4 (sense or antisense). Transformed soybean plants are identified by selection on media containing glyphosate. Fatty acid compositions are analyzed from seed of soybean lines transformed with the constructs using gas chromatography. Seed of the transformed plants exhibit high levels of oleic acid (over 80%).